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The Microbial World and You

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The overall theme of this textbook is the relationship between microbes (very small organisms that usually require a microscope to be seen) and our lives. This relationship involves not only the familiar harmful effects of certain microorganisms, such as disease and food spoilage, but also their many beneficial effects. In this chapter we introduce you to some of the many ways microbes affect our lives. Microbes have been fruitful subjects of study for many years. We begin by introducing you to how organisms are named and classified, followed by a short history of microbiology that reveals how much we have learned in just a few hundred years. We then discuss the incredible diversity of microorganisms and their ecological importance, noting how they maintain balance in the environment by recycling chemical elements such as carbon and nitrogen among the soil, organisms, and the atmosphere. We also examine how microbes are used in commercial and industrial applications to produce foods, chemicals, and drugs (such as antibiotics); and to treat sewage, control pests, and clean up pollutants. We will discuss microbes as the cause of such diseases as avian (bird) flu, West Nile encephalitis, mad cow disease, diarrhea, hemorrhagic fever, and AIDS. We will also examine the growing public health problem of antibiotic-resistant bacteria. *Staphylococcus aureus* bacteria on human nasal epithelial cells are shown in the photograph. These bacteria live harmlessly on skin or inside the nose. Misuse of antibiotics allows the survival of bacteria with antibiotic-resistant genes such as methicillin-resistant *S. aureus* (MRSA). As illustrated in the Clinical Case, an infection caused by these bacteria is resistant to antibiotic treatment.

Microbes in Our Lives

LEARNING OBJECTIVE

1-1 List several ways in which microbes affect our lives.

For many people, the words *germ* and *microbe* bring to mind a group of tiny creatures that do not quite fit into any of the categories in that old question, “Is it animal, vegetable, or mineral?” **Microbes**, also called **microorganisms**, are minute living things that individually are usually too small to be seen with the unaided eye. The group includes bacteria (Chapter 11), fungi (yeasts and molds), protozoa, and microscopic algae (Chapter 12). It also includes viruses, those noncellular entities sometimes regarded as straddling the border between life and nonlife (Chapter 13). You will be introduced to each of these groups of microbes shortly.

We tend to associate these small organisms only with major diseases such as AIDS, uncomfortable infections, or such common inconveniences as spoiled food. However, the majority of microorganisms actually help maintain the balance of living organisms and chemicals in our environment. Marine and freshwater microorganisms form the basis of the food chain in oceans, lakes, and rivers. Soil microbes help break down wastes and incorporate nitrogen gas from the air into organic compounds, thereby recycling chemical elements between the soil, water, life, and air. Certain microbes play important roles in *photosynthesis*, a food- and oxygen-generating process that is critical to life on Earth. Humans and many other animals depend on the microbes in their intestines for digestion and the synthesis of some vitamins that their bodies require, including some B vitamins for metabolism and vitamin K for blood clotting.

Microorganisms also have many commercial applications. They are used in the synthesis of such chemical products as

vitamins, organic acids, enzymes, alcohols, and many drugs. For example, microbes are used to produce acetone and butanol, and the vitamins B₂ (riboflavin) and B₁₂ (cobalamin) are made biochemically. The process by which microbes produce acetone and butanol was discovered in 1914 by Chaim Weizmann, a Russian-born chemist working in England. With the outbreak of World War I in August of that year, the production of acetone became very important for making cordite (a smokeless form of gunpowder used in munitions). Weizmann’s discovery played a significant role in determining the outcome of the war.

The food industry also uses microbes in producing, for example, vinegar, sauerkraut, pickles, soy sauce, cheese, yogurt, bread, and alcoholic beverages. In addition, enzymes from microbes can now be manipulated to cause the microbes to produce substances they normally do not synthesize, including cellulose, digestive aids, and drain cleaner, plus important therapeutic substances such as insulin. Microbial enzymes may even have helped produce your favorite pair of jeans (see the box on page 3).

Though only a minority of microorganisms are **pathogenic** (disease-producing), practical knowledge of microbes is necessary for medicine and the related health sciences. For example, hospital workers must be able to protect patients from common microbes that are normally harmless but pose a threat to the sick and injured.

Today we understand that microorganisms are found almost everywhere. Yet not long ago, before the invention of the microscope, microbes were unknown to scientists. Thousands of people died in devastating epidemics, the causes of which were not understood. Entire families died because vaccinations and antibiotics were not available to fight infections.

We can get an idea of how our current concepts of microbiology developed by looking at a few historic milestones in microbiology that have changed our lives. First, however, we will look at the major groups of microbes and how they are named and classified.

CHECK YOUR UNDERSTANDING

- ✓ Describe some of the destructive and beneficial actions of microbes. **1-1***

Naming and Classifying Microorganisms

LEARNING OBJECTIVES

- 1-2** Recognize the system of scientific nomenclature that uses two names: a genus and a specific epithet.
- 1-3** Differentiate the major characteristics of each group of microorganisms.
- 1-4** List the three domains.

* The numbers following Check Your Understanding questions refer to the corresponding Learning Objectives.

Clinical Case: A Simple Spider Bite?

Andrea is a normally healthy 22-year-old college student who lives at home with her mother and younger sister, a high school gymnast. She is trying to work on a paper for her psychology class but is having a hard time because a red, swollen sore on her right wrist is making typing difficult. “Why won’t this spider bite heal?” she wonders. “It’s been there for days!” She makes an appointment with her doctor so she can show him the painful lesion. Although Andrea does not have a fever, she does have an elevated white blood cell count that indicates a bacterial infection. Andrea’s doctor suspects that this isn’t a spider bite at all, but a staph infection. He prescribes a β -lactam antibiotic, cephalosporin. Learn more about the development of Andrea’s illness on the following pages.

What is staph? Read on to find out.

2 17 19 20 21

Designer Jeans: Made by Microbes?

Denim blue jeans have become increasingly popular ever since Levi Strauss and Jacob Davis first made them for California gold miners in 1873. Now, companies that manufacture blue jeans are turning to microbiology to develop environmentally sound production methods that minimize toxic wastes and the associated costs.

Stone Washing?

A softer denim, called "stone-washed," was introduced in the 1980s. Enzymes, called cellulases, from *Trichoderma* fungus are used to digest some of the cellulose in the cotton, thereby softening it and giving the stone-washed appearance. Unlike many chemical reactions, enzymes usually operate at safe temperatures and pH. Moreover, enzymes are proteins, so they are readily degraded for removal from wastewater.

Fabric

Cotton production requires large tracts of land, pesticides, and fertilizer, and the crop yield depends on the weather. However, bacteria can produce both cotton and polyester with less environmental impact. *Gluconacetobacter xylinus* bacteria make cellulose by attaching glucose units to simple chains in the outer membrane of the bacterial cell wall. The cellulose microfibrils are extruded through pores in the outer

membrane, and bundles of microfibrils then twist into ribbons.

Bleaching

Peroxide is a safer bleaching agent than chlorine and can be easily removed from fabric and wastewater by enzymes. Researchers at Novo Nordisk Biotech cloned a mushroom peroxidase gene in yeast and grew the yeasts in washing machine conditions. The yeast that survived the washing machine were selected as the peroxidase producers.

Indigo

Chemical synthesis of indigo requires a high pH and produces waste that explodes in contact with air. However, a California biotechnology company, Genencor, has developed a method to produce indigo by using bacteria. Researchers identified a gene from a soil bacterium, *Pseudomonas putida*, for conversion of the bacterial by-product indole to indigo. This gene was put into *Escherichia coli* bacteria, which then turned blue.

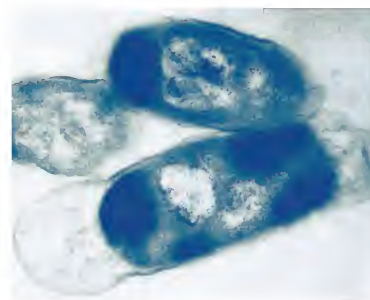
Bioplastic

Microbes can even make plastic zippers and packaging

material for the jeans. Over 25 bacteria make polyhydroxyalkanoate (PHA) inclusion granules as a food reserve. PHAs are similar to common plastics, and because they are made by bacteria, they are also readily degraded by many bacteria. PHAs could provide a biodegradable alternative to conventional plastic, which is made from petroleum.



E. coli bacteria produce indigo from tryptophan.



Indigo-producing *E. coli* bacteria.

0.3 μm

TEM

Nomenclature

The system of nomenclature (naming) for organisms in use today was established in 1735 by Carolus Linnaeus. Scientific names are latinized because Latin was the language traditionally used by scholars. Scientific nomenclature assigns each organism two names—the **genus** (plural: *genera*) is the first name and is always capitalized; the **specific epithet** (*species* name) follows and is not capitalized. The organism is referred to by both the genus and the specific epithet, and both names are underlined or italicized. By custom, after a scientific name has been mentioned once, it can be abbreviated with the initial of the genus followed by the specific epithet.

Scientific names can, among other things, describe an organism, honor a researcher, or identify the habitat of a species. For example, consider *Staphylococcus aureus* (staf-i-lō-kok'kus ô'rē-us), a bacterium commonly found on human skin. *Staphylo-* describes the clustered arrangement of the cells; *coccus* indicates that they

are shaped like spheres. The specific epithet, *aureus*, is Latin for golden, the color of many colonies of this bacterium. The genus of the bacterium *Escherichia coli* (esh-ë-rik'-ë-ä kō'li or kō'lē) is named for a scientist, Theodor Escherich, whereas its specific epithet, *coli*, reminds us that *E. coli* live in the colon, or large intestine. **Table 1.1** contains more examples.

CHECK YOUR UNDERSTANDING

✓ Distinguish a genus from a specific epithet. **1-2**

Types of Microorganisms

The classification and identification of microorganisms is discussed in Chapter 10. Here is an overview of the major groups.

Bacteria

Bacteria (singular: **bacterium**) are relatively simple, single-celled (unicellular) organisms. Because their genetic material is not

TABLE 1.1 Making Scientific Names Familiar

Use the word roots guide in Appendix E to find out what the name means. The name will not seem so strange if you translate it. When you encounter a new name, practice saying it out loud. The exact pronunciation is not as important as the familiarity you will gain. Guidelines for pronunciation are given in Appendix D.

Following are some examples of microbial names you may encounter in the popular press as well as in the lab.

	Pronunciation	Source of Genus Name	Source of Specific Epithet
<i>Salmonella enterica</i> (bacterium)	sal-mōn-el'lä en-ter'i-kä	Honors public health microbiologist Daniel Salmon	Found in the intestines (<i>entero</i> -)
<i>Streptococcus pyogenes</i> (bacterium)	strep-tō-kok'kus pl-āj'en-ēz	Appearance of cells in chains (<i>strepto</i> -)	Forms pus (<i>pyo</i> -)
<i>Saccharomyces cerevisiae</i> (yeast)	sak-ä-rō-mī'ses se-ri-vis'ē-tī	Fungus (<i>-myces</i>) that uses sugar (<i>saccharo</i> -)	Makes beer (<i>cerevisia</i>)
<i>Penicillium chrysogenum</i> (fungus)	pen-i-sil'lē-um krī-so'jen-um	Tuftlike or paintbrush (<i>penicill</i> -) appearance microscopically	Produces a yellow (<i>chryso</i> -) pigment
<i>Trypanosoma cruzi</i> (protozoan)	tri-pa-nō-sō'mä krüz'ē	Corkscrew- (<i>trypano</i> -, borer; <i>soma</i> -, body)	Honors epidemiologist Oswaldo Cruz

enclosed in a special nuclear membrane, bacterial cells are called **prokaryotes** (prō-kar'e-ōts), from Greek words meaning prenucleus. Prokaryotes include both bacteria and archaea.

Bacterial cells generally appear in one of several shapes. *Bacillus* (bā-sil'lus) (rodlike), illustrated in **Figure 1.1a**, *coccus* (kok'kus) (spherical or ovoid), and *spiral* (corkscrew or curved) are among the most common shapes, but some bacteria are star-shaped or square (see Figures 4.1 through 4.5, pages 77–78). Individual bacteria may form pairs, chains, clusters, or other groupings; such formations are usually characteristic of a particular genus or species of bacteria.

Bacteria are enclosed in cell walls that are largely composed of a carbohydrate and protein complex called *peptidoglycan*. (By contrast, cellulose is the main substance of plant and algal cell walls.) Bacteria generally reproduce by dividing into two equal cells; this process is called *binary fission*. For nutrition, most bacteria use organic chemicals, which in nature can be derived from either dead or living organisms. Some bacteria can manufacture their own food by photosynthesis, and some can derive nutrition from inorganic substances. Many bacteria can “swim” by using moving appendages called *flagella*. (For a complete discussion of bacteria, see Chapter 11.)

Archaea

Like bacteria, **archaea** (är'kē-ä) consist of prokaryotic cells, but if they have cell walls, the walls lack peptidoglycan. Archaea, often found in extreme environments, are divided into three main groups. The *methanogens* produce methane as a waste product from respiration. The *extreme halophiles* (*halo* = salt; *philic* = loving) live in extremely salty environments such as the Great Salt Lake and the Dead Sea. The *extreme thermophiles*

(*therm* = heat) live in hot sulfurous water, such as hot springs at Yellowstone National Park. Archaea are not known to cause disease in humans.

Fungi

Fungi (singular: **fungus**) are **eukaryotes** (yū-kar'ē-ōts), organisms whose cells have a distinct nucleus containing the cell's genetic material (DNA), surrounded by a special envelope called the nuclear membrane. Organisms in the Kingdom Fungi may be unicellular or multicellular (see Chapter 12, page 331). Large multicellular fungi, such as mushrooms, may look somewhat like plants, but unlike most plants, fungi cannot carry out photosynthesis. True fungi have cell walls composed primarily of a substance called *chitin*. The unicellular forms of fungi, *yeasts*, are oval microorganisms that are larger than bacteria. The most typical fungi are *molds* (**Figure 1.1b**). Molds form visible masses called *mycelia*, which are composed of long filaments (*hyphae*) that branch and intertwine. The cottony growths sometimes found on bread and fruit are mold mycelia. Fungi can reproduce sexually or asexually. They obtain nourishment by absorbing solutions of organic material from their environment—whether soil, seawater, freshwater, or an animal or plant host. Organisms called *slime molds* have characteristics of both fungi and amoebas. They are discussed in detail in Chapter 12.

Protozoa

Protozoa (singular: **protozoan**) are unicellular eukaryotic microbes (see Chapter 12, page 348). Protozoa move by pseudopods, flagella, or cilia. Amebae (**Figure 1.1c**) move by using extensions of their cytoplasm called *pseudopods* (false feet). Other protozoa have long *flagella* or numerous shorter appendages for locomotion

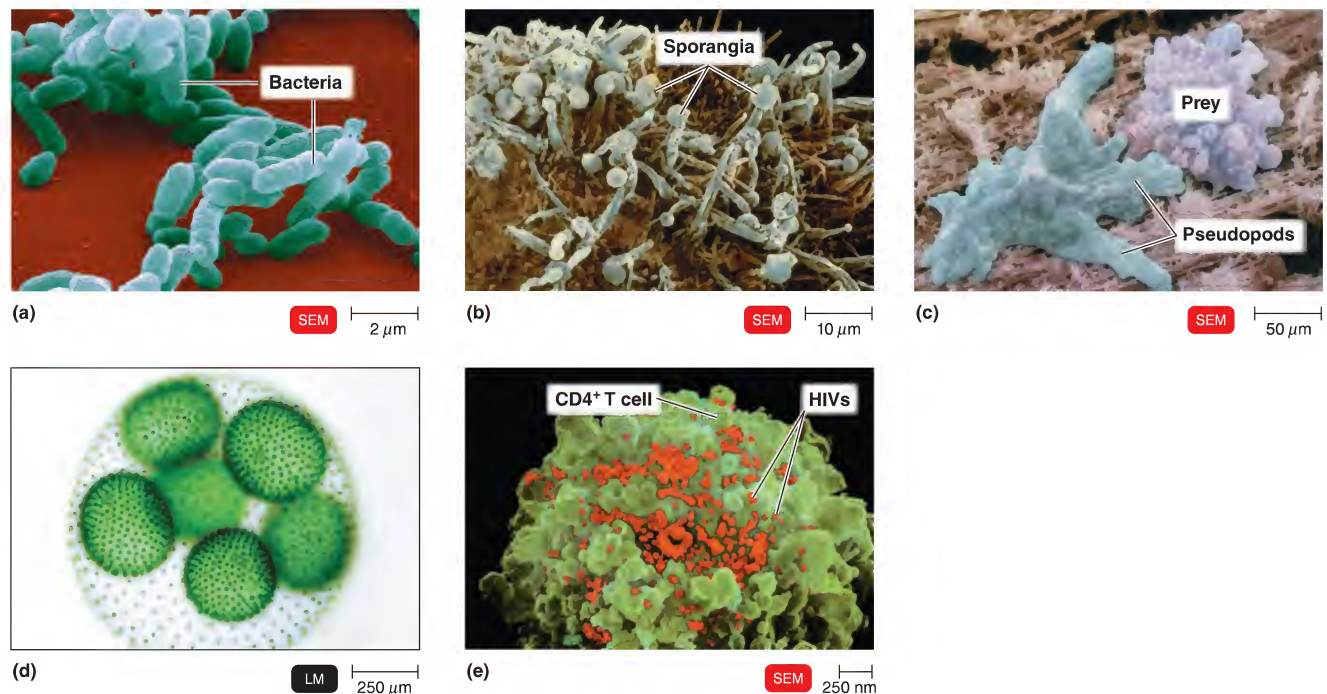


Figure 1.1 Types of microorganisms.

NOTE: Throughout the book, a red icon under a micrograph indicates that the micrograph has been artificially colored. (a) The rod-shaped bacterium *Haemophilus influenzae*, one of the bacterial causes of pneumonia. (b) *Mucor*, a

common bread mold, is a type of fungus. When released from sporangia, spores that land on a favorable surface germinate into a network of hyphae (filaments) that absorb nutrients. (c) An amoeba, a protozoan, approaching a food particle. (d) The pond alga *Volvox*. (e) Several human

immunodeficiency viruses (HIVs), the causative agent of AIDS, budding from a CD4⁺ T cell.

Q How are bacteria, archaea, fungi, protozoa, algae, and viruses distinguished on the basis of cellular structure?

called *cilia*. Protozoa have a variety of shapes and live either as free entities or as *parasites* (organisms that derive nutrients from living hosts) that absorb or ingest organic compounds from their environment. Some protozoa, such as *Euglena*, are photosynthetic. They use light as a source of energy and carbon dioxide as their chief source of carbon to produce sugars. Protozoa can reproduce sexually or asexually.

Algae

Algae (singular: **alga**) are photosynthetic eukaryotes with a wide variety of shapes and both sexual and asexual reproductive forms (Figure 1.1d). The algae of interest to microbiologists are usually unicellular (see Chapter 12, page 343). The cell walls of many algae are composed of a carbohydrate called *cellulose*. Algae are abundant in freshwater and salt water, in soil, and in association with plants. As photosynthesizers, algae need light, water, and carbon dioxide for food production and growth, but they do not generally require organic compounds from the environment. As a result of photosynthesis, algae produce oxygen and carbohydrates that are then utilized by other organisms, including animals. Thus, they play an important role in the balance of nature.

Viruses

Viruses (Figure 1.1e) are very different from the other microbial groups mentioned here. They are so small that most can be seen only with an electron microscope, and they are acellular (not cellular). Structurally very simple, a virus particle contains a core made of only one type of nucleic acid, either DNA or RNA. This core is surrounded by a protein coat, which is sometimes encased by a lipid membrane called an envelope. All living cells have RNA *and* DNA, can carry out chemical reactions, and can reproduce as self-sufficient units. Viruses can reproduce only by using the cellular machinery of other organisms. Thus, on the one hand, viruses are considered to be living only when they multiply within host cells they infect. In this sense, viruses are parasites of other forms of life. On the other hand, viruses are not considered to be living because they are inert outside living hosts. (Viruses will be discussed in detail in Chapter 13.)

Multicellular Animal Parasites

Although multicellular animal parasites are not strictly microorganisms, they are of medical importance and therefore will be

discussed in this text. Animal parasites are eukaryotes. The two major groups of parasitic worms are the flatworms and the roundworms, collectively called **helminths** (see Chapter 12, page 354). During some stages of their life cycle, helminths are microscopic in size. Laboratory identification of these organisms includes many of the same techniques used for identifying microbes.

CHECK YOUR UNDERSTANDING

- ✓ Which groups of microbes are prokaryotes? Which are eukaryotes? **1-3**

Classification of Microorganisms

Before the existence of microbes was known, all organisms were grouped into either the animal kingdom or the plant kingdom. When microscopic organisms with characteristics of animals and plants were discovered late in the seventeenth century, a new system of classification was needed. Still, biologists could not agree on the criteria for classifying these new organisms until the late 1970s.

In 1978, Carl Woese devised a system of classification based on the cellular organization of organisms. It groups all organisms in three domains as follows:

1. Bacteria (cell walls contain a protein–carbohydrate complex called peptidoglycan)
2. Archaea (cell walls, if present, lack peptidoglycan)
3. Eukarya, which includes the following:
 - Protists (slime molds, protozoa, and algae)
 - Fungi (unicellular yeasts, multicellular molds, and mushrooms)
 - Plants (mosses, ferns, conifers, and flowering plants)
 - Animals (sponges, worms, insects, and vertebrates)

Classification will be discussed in more detail in Chapters 10 through 12.

CHECK YOUR UNDERSTANDING

- ✓ What are the three domains? **1-4**

A Brief History of Microbiology

LEARNING OBJECTIVES

- 1-5** Explain the importance of observations made by Hooke and van Leeuwenhoek.
- 1-6** Compare spontaneous generation and biogenesis.
- 1-7** Identify the contributions to microbiology made by Needham, Spallanzani, Virchow, and Pasteur.
- 1-8** Explain how Pasteur's work influenced Lister and Koch.
- 1-9** Identify the importance of Koch's postulates.
- 1-10** Identify the importance of Jenner's work.
- 1-11** Identify the contributions to microbiology made by Ehrlich and Fleming.

- 1-12** Define *bacteriology*, *mycology*, *parasitology*, *immunology*, and *virology*.

- 1-13** Explain the importance of microbial genetics and molecular biology.

The science of microbiology dates back only 200 years, yet the recent discovery of *Mycobacterium tuberculosis* (mī-kō-bak-ti'rē-um tū-bēr-ku-lō'sis) DNA in 3000-year-old Egyptian mummies reminds us that microorganisms have been around for much longer. In fact, bacterial ancestors were the first living cells to appear on Earth. Although we know relatively little about what earlier people thought about the causes, transmission, and treatment of disease, we know more about the history of the past few hundred years. Let's look now at some key developments in microbiology that have spurred the field to its current technological state.

The First Observations

One of the most important discoveries in biology occurred in 1665. After observing a thin slice of cork through a relatively crude microscope, an Englishman, Robert Hooke, reported to the world that life's smallest structural units were "little boxes," or "cells," as he called them. Using his improved version of a compound microscope (one that uses two sets of lenses), Hooke was able to see individual cells. Hooke's discovery marked the beginning of the **cell theory**—the theory that *all living things are composed of cells*. Subsequent investigations into the structure and function of cells were based on this theory.

Though Hooke's microscope was capable of showing large cells, it lacked the resolution that would have allowed him to see microbes clearly. The Dutch merchant and amateur scientist Anton van Leeuwenhoek was probably the first actually to observe live microorganisms through the magnifying lenses of more than 400 microscopes he constructed. Between 1673 and 1723, he wrote a series of letters to the Royal Society of London describing the "animalcules" he saw through his simple, single-lens microscope. Van Leeuwenhoek made detailed drawings of "animalcules" he found in rainwater, in his own feces, and in material scraped from his teeth. These drawings have since been identified as representations of bacteria and protozoa (**Figure 1.2**).

CHECK YOUR UNDERSTANDING

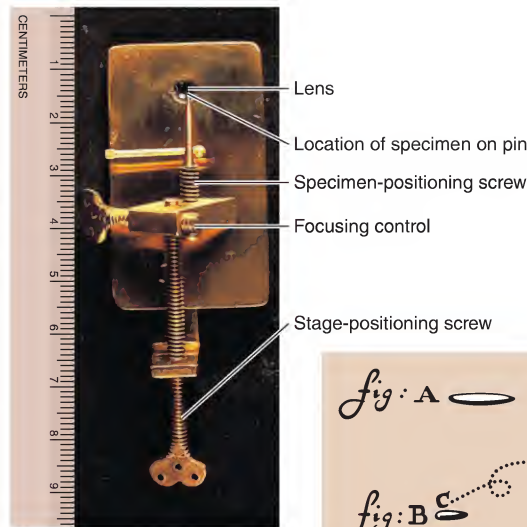
- ✓ What is the cell theory? **1-5**

The Debate over Spontaneous Generation

After van Leeuwenhoek discovered the previously "invisible" world of microorganisms, the scientific community of the time became interested in the origins of these tiny living things. Until the second half of the nineteenth century, many scientists and philosophers believed that some forms of life could arise spontaneously from nonliving matter; they called this hypothetical process **spontaneous generation**. Not much more than 100 years ago, people commonly believed that toads, snakes, and mice could be born of moist soil; that flies could emerge from manure;



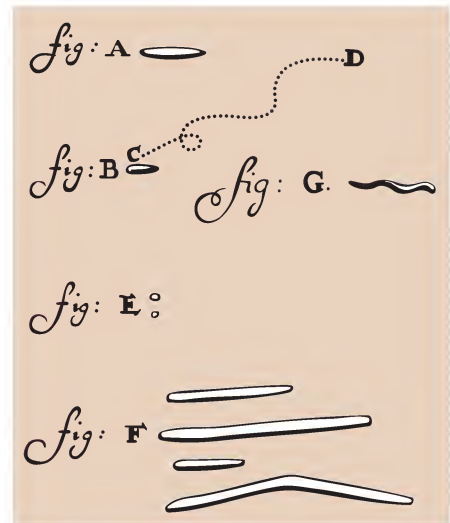
(a) Van Leeuwenhoek using his microscope



(b) Microscope replica

Figure 1.2 Anton van Leeuwenhoek's microscopic observations. (a) By holding his brass microscope toward a source of light, van Leeuwenhoek was able to observe living organisms too small to be seen with the unaided eye. (b) The specimen was placed on the tip of the adjustable point and viewed from the other side through the tiny, nearly spherical lens. The highest magnification possible with his microscopes was about 300 \times (times). (c) Some of van Leeuwenhoek's drawings of bacteria, made in 1683. The letters represent various shapes of bacteria. C–D represents a path of motion he observed.

Q Why was van Leeuwenhoek's discovery so important?



(c) Drawings of bacteria

and that maggots (which we now know are the larvae of flies) could arise from decaying corpses.

Evidence Pro and Con

A strong opponent of spontaneous generation, the Italian physician Francesco Redi set out in 1668 to demonstrate that maggots did not arise spontaneously from decaying meat. Redi filled two jars with decaying meat. The first was left unsealed; the flies laid their eggs on the meat, and the eggs developed into larvae. The second jar was sealed, and because the flies could not lay their eggs on the meat, no maggots appeared. Still, Redi's antagonists were not convinced; they claimed that fresh air was needed for spontaneous generation. So Redi set up a second experiment, in which he covered a jar with a fine net instead of sealing it. No larvae appeared in the gauze-covered jar, even though air was present. Maggots appeared only when flies were allowed to leave their eggs on the meat.

Redi's results were a serious blow to the long-held belief that large forms of life could arise from nonlife. However, many scientists still believed that small organisms, such as

van Leeuwenhoek's "animalcules," were simple enough to be generated from nonliving materials.

The case for spontaneous generation of microorganisms seemed to be strengthened in 1745, when John Needham, an Englishman, found that even after he heated nutrient fluids (chicken broth and corn broth) before pouring them into covered flasks, the cooled solutions were soon teeming with microorganisms. Needham claimed that microbes developed spontaneously from the fluids. Twenty years later, Lazzaro Spallanzani, an Italian scientist, suggested that microorganisms from the air probably had entered Needham's solutions after they were boiled. Spallanzani showed that nutrient fluids heated *after* being sealed in a flask did not develop microbial growth. Needham responded by claiming the "vital force" necessary for spontaneous generation had been destroyed by the heat and was kept out of the flasks by the seals.

This intangible "vital force" was given all the more credence shortly after Spallanzani's experiment, when Anton Laurent Lavoisier showed the importance of oxygen to life. Spallanzani's observations were criticized on the grounds that there was not enough oxygen in the sealed flasks to support microbial life.

The Theory of Biogenesis

The issue was still unresolved in 1858, when the German scientist Rudolf Virchow challenged the case for spontaneous generation with the concept of **biogenesis**, the claim that living cells can arise only from preexisting living cells. Because he could offer no scientific proof, arguments about spontaneous generation continued until 1861, when the issue was finally resolved by the French scientist Louis Pasteur.

With a series of ingenious and persuasive experiments, Pasteur demonstrated that microorganisms are present in the air and can contaminate sterile solutions, but that air itself does not create microbes. He filled several short-necked flasks with beef broth and then boiled their contents. Some were then left open and allowed to cool. In a few days, these flasks were found to be contaminated with microbes. The other flasks, sealed after boiling, were free of microorganisms. From these results, Pasteur reasoned that microbes in the air were the agents responsible for contaminating nonliving matter.

Pasteur next placed broth in open-ended, long-necked flasks and bent the necks into S-shaped curves (**Figure 1.3**). The contents of these flasks were then boiled and cooled. The broth in the flasks did not decay and showed no signs of life, even after months. Pasteur's unique design allowed air to pass into the flask, but the curved neck trapped any airborne microorganisms that might contaminate the broth. (Some of these original vessels are still on display at the Pasteur Institute in Paris. They have been sealed but, like the flask shown in **Figure 1.3**, show no sign of contamination more than 100 years later.)

Pasteur showed that microorganisms can be present in nonliving matter—on solids, in liquids, and in the air. Furthermore, he demonstrated conclusively that microbial life can be destroyed by heat and that methods can be devised to block the access of airborne microorganisms to nutrient environments. These discoveries form the basis of **aseptic techniques**, techniques that prevent contamination by unwanted microorganisms, which are now the standard practice in laboratory and many medical procedures. Modern aseptic techniques are among the first and most important concepts that a beginning microbiologist learns.

Pasteur's work provided evidence that microorganisms cannot originate from mystical forces present in nonliving materials. Rather, any appearance of “spontaneous” life in nonliving solutions can be attributed to microorganisms that were already present in the air or in the fluids themselves. Scientists now believe that a form of spontaneous generation probably did occur on the primitive Earth when life first began, but they agree that this does not happen under today's environmental conditions.

CHECK YOUR UNDERSTANDING

- What evidence supported spontaneous generation? **1-6**
- How was spontaneous generation disproved? **1-7**

The Golden Age of Microbiology

The work that began with Pasteur started an explosion of discoveries in microbiology. The period from 1857 to 1914 has been appropriately named the Golden Age of Microbiology. During this period, rapid advances, spearheaded mainly by Pasteur and Robert Koch, led to the establishment of microbiology as a science. Discoveries during these years included both the agents of many diseases and the role of immunity in preventing and curing disease. During this productive period, microbiologists studied the chemical activities of microorganisms, improved the techniques for performing microscopy and culturing microorganisms, and developed vaccines and surgical techniques. Some of the major events that occurred during the Golden Age of Microbiology are listed in **Figure 1.4**.

Fermentation and Pasteurization

One of the key steps that established the relationship between microorganisms and disease occurred when a group of French merchants asked Pasteur to find out why wine and beer soured. They hoped to develop a method that would prevent spoilage when those beverages were shipped long distances. At the time, many scientists believed that air converted the sugars in these fluids into alcohol. Pasteur found instead that microorganisms called yeasts convert the sugars to alcohol in the absence of air. This process, called **fermentation** (see Chapter 5, page 130), is used to make wine and beer. Souring and spoilage are caused by different microorganisms called bacteria. In the presence of air, bacteria change the alcohol into vinegar (acetic acid).

Pasteur's solution to the spoilage problem was to heat the beer and wine just enough to kill most of the bacteria that caused the spoilage. The process, called **pasteurization**, is now commonly used to reduce spoilage and kill potentially harmful bacteria in milk as well as in some alcoholic drinks. Showing the connection between food spoilage and microorganisms was a major step toward establishing the relationship between disease and microbes.

The Germ Theory of Disease

As we have seen, the fact that many kinds of diseases are related to microorganisms was unknown until relatively recently. Before the time of Pasteur, effective treatments for many diseases were discovered by trial and error, but the causes of the diseases were unknown.

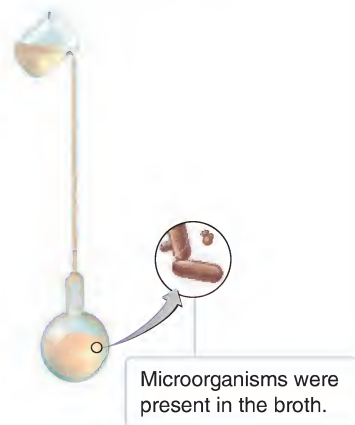
The realization that yeasts play a crucial role in fermentation was the first link between the activity of a microorganism and physical and chemical changes in organic materials. This discovery alerted scientists to the possibility that microorganisms might have similar relationships with plants and animals—specifically, that microorganisms might cause disease. This idea was known as the **germ theory of disease**.

FOUNDATION FIGURE 1.3

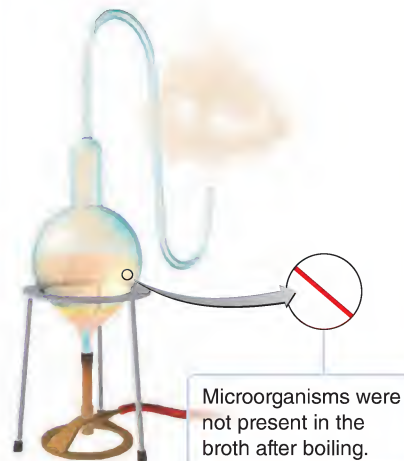
Disproving the Theory of Spontaneous Generation

According to the theory of spontaneous generation, life can arise spontaneously from nonliving matter, such as dead corpses and soil. Pasteur's experiment, described below, demonstrated that microbes are present in nonliving matter—air, liquids, and solids.

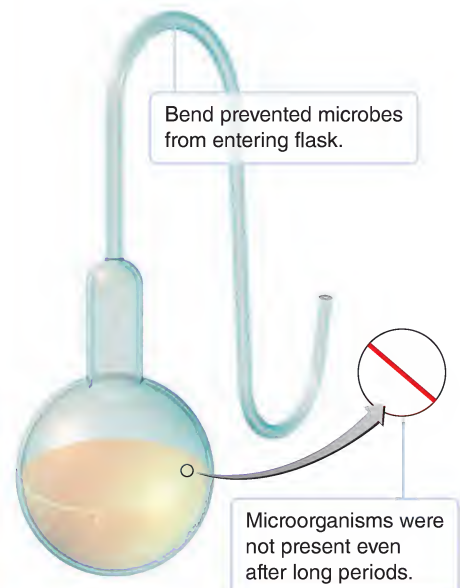
- 1 Pasteur first poured beef broth into a long-necked flask.



- 2 Next he heated the neck of the flask and bent it into an S-shape; then he boiled the broth for several minutes.



- 3 Microorganisms did not appear in the cooled solution, even after long periods.



KEY CONCEPTS

- Pasteur demonstrated that microbes are responsible for food spoilage, leading researchers to the connection between microbes and disease.
- His experiments and observations provided the basis of aseptic techniques, which are used to prevent microbial contamination, as shown in the photo at right.

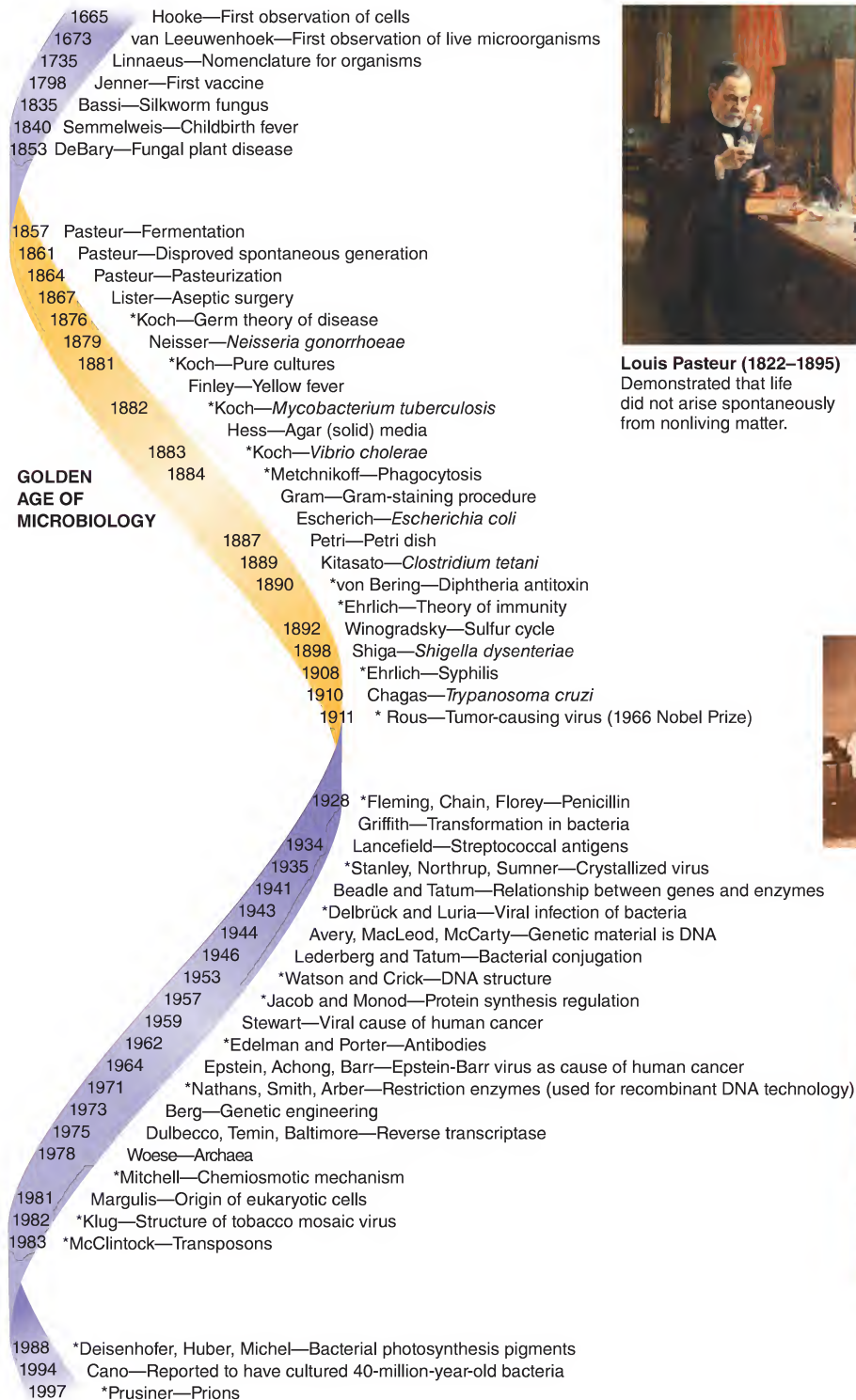


The germ theory was a difficult concept for many people to accept at that time because for centuries disease was believed to be punishment for an individual's crimes or misdeeds. When the inhabitants of an entire village became ill, people often blamed the disease on demons appearing as foul odors from sewage or on poisonous vapors from swamps. Most people born in Pasteur's time found it inconceivable that "invisible" microbes could travel through the air to infect plants and animals or remain on clothing and bedding to be transmitted from one person to another. Despite these doubts scientists gradually accumulated the information needed to support the new germ theory.

In 1865, Pasteur was called upon to help fight silkworm disease, which was ruining the silk industry throughout Europe.

Years earlier, in 1835, Agostino Bassi, an amateur microscopist, had proved that another silkworm disease was caused by a fungus. Using data provided by Bassi, Pasteur found that the more recent infection was caused by a protozoan, and he developed a method for recognizing afflicted silkworm moths.

In the 1860s, Joseph Lister, an English surgeon, applied the germ theory to medical procedures. Lister was aware that in the 1840s, the Hungarian physician Ignaz Semmelweis had demonstrated that physicians, who at the time did not disinfect their hands, routinely transmitted infections (puerperal, or child-birth, fever) from one obstetrical patient to another. Lister had also heard of Pasteur's work connecting microbes to animal diseases. Disinfectants were not used at the time, but Lister knew



Louis Pasteur (1822–1895)
Demonstrated that life did not arise spontaneously from nonliving matter.



Robert Koch (1843–1910)
Established experimental steps for directly linking a specific microbe to a specific disease.



Joseph Lister (1827–1912)
Performed surgery under antiseptic conditions using phenol. Proved that microbes caused surgical wound infections.



Rebecca C. Lancefield (1895–1981)
Classified streptococci according to serotypes (variants within a species)

Figure 1.4 Milestones in microbiology, highlighting those that occurred during the Golden Age of Microbiology. An asterisk (*) indicates a Nobel laureate.

Q Why do you think the Golden Age of Microbiology occurred when it did?

that phenol (carbolic acid) kills bacteria, so he began treating surgical wounds with a phenol solution. The practice so reduced the incidence of infections and deaths that other surgeons quickly adopted it. Lister's technique was one of the earliest medical attempts to control infections caused by microorganisms. In fact, his findings proved that microorganisms cause surgical wound infections.

The first proof that bacteria actually cause disease came from Robert Koch in 1876. Koch, a German physician, was Pasteur's young rival in the race to discover the cause of anthrax, a disease that was destroying cattle and sheep in Europe. Koch discovered rod-shaped bacteria now known as *Bacillus anthracis* (bă-sil'lus an-thră'sis) in the blood of cattle that had died of anthrax. He cultured the bacteria on nutrients and then injected samples of the culture into healthy animals. When these animals became sick and died, Koch isolated the bacteria in their blood and compared them with the originally isolated bacteria. He found that the two sets of blood cultures contained the same bacteria.

Koch thus established **Koch's postulates**, a sequence of experimental steps for directly relating a specific microbe to a specific disease (see Figure 14.3, page 407). During the past 100 years, these same criteria have been invaluable in investigations proving that specific microorganisms cause many diseases. Koch's postulates, their limitations, and their application to disease will be discussed in greater detail in Chapter 14.

Vaccination

Often a treatment or preventive procedure is developed before scientists know why it works. The smallpox vaccine is an example. On May 4, 1796, almost 70 years before Koch established that a specific microorganism causes anthrax, Edward Jenner, a young British physician, embarked on an experiment to find a way to protect people from smallpox.

Smallpox epidemics were greatly feared. The disease periodically swept through Europe, killing thousands, and it wiped out 90% of the American Indians on the East Coast when European settlers first brought the infection to the New World.

When a young milkmaid informed Jenner that she couldn't get smallpox because she already had been sick from cowpox—a much milder disease—he decided to put the girl's story to the test. First Jenner collected scrapings from cowpox blisters. Then he inoculated a healthy 8-year-old volunteer with the cowpox material by scratching the person's arm with a pox-contaminated needle. The scratch turned into a raised bump. In a few days, the volunteer became mildly sick but recovered and never again contracted either cowpox or smallpox. The process was called **vaccination**, from the Latin word *vacca*, meaning cow. Pasteur gave it this name in honor of Jenner's work. The protection from disease provided by vaccination (or by recovery from the disease

itself) is called **immunity**. We will discuss the mechanisms of immunity in Chapter 17.

Years after Jenner's experiment, in about 1880, Pasteur discovered why vaccinations work. He found that the bacterium that causes fowl cholera lost its ability to cause disease (lost its *virulence*, or became *avirulent*) after it was grown in the laboratory for long periods. However, it—and other microorganisms with decreased virulence—was able to induce immunity against subsequent infections by its virulent counterparts. The discovery of this phenomenon provided a clue to Jenner's successful experiment with cowpox. Both cowpox and smallpox are caused by viruses. Even though cowpox virus is not a laboratory-produced derivative of smallpox virus, it is so closely related to the smallpox virus that it can induce immunity to both viruses. Pasteur used the term *vaccine* for cultures of avirulent microorganisms used for preventive inoculation.

Jenner's experiment marked the first time in a Western culture that a living viral agent—the cowpox virus—was used to produce immunity. Physicians in China had immunized patients from smallpox by removing scales from drying pustules of a person suffering from a mild case of smallpox, grinding the scales to a fine powder, and inserting the powder into the nose of the person to be protected.

Some vaccines are still produced from avirulent microbial strains that stimulate immunity to the related virulent strain. Other vaccines are made from killed virulent microbes, from isolated components of virulent microorganisms, or by genetic engineering techniques.

CHECK YOUR UNDERSTANDING

- Summarize in your own words the germ theory of disease. **1-8**
- What is the importance of Koch's postulates? **1-9**
- What is the significance of Jenner's discovery? **1-10**

The Birth of Modern Chemotherapy: Dreams of a "Magic Bullet"

After the relationship between microorganisms and disease was established, medical microbiologists next focused on the search for substances that could destroy pathogenic microorganisms without damaging the infected animal or human. Treatment of disease by using chemical substances is called **chemotherapy**. (The term also commonly refers to chemical treatment of non-infectious diseases, such as cancer.) Chemicals produced naturally by bacteria and fungi to act against other microorganisms are called **antibiotics**. Chemotherapeutic agents prepared from chemicals in the laboratory are called **synthetic drugs**. The success of chemotherapy is based on the fact that some chemicals are more poisonous to microorganisms than to the hosts infected by the microbes. Antimicrobial therapy will be discussed in further detail in Chapter 20.

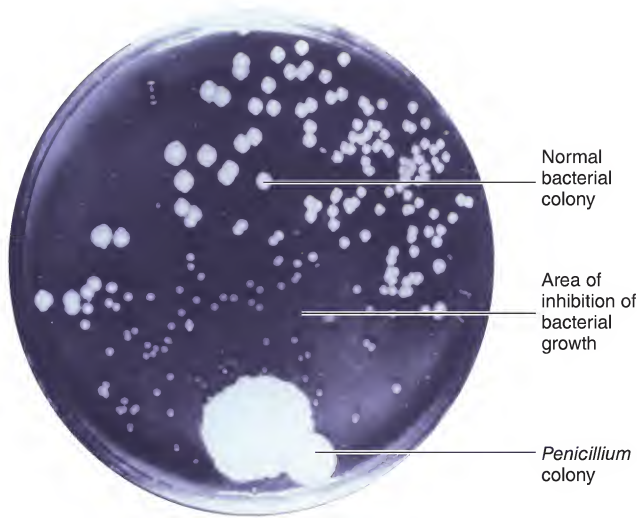


Figure 1.5 The discovery of penicillin. Alexander Fleming took this photograph in 1928. The colony of *Penicillium* mold accidentally contaminated the plate and inhibited nearby bacterial growth.

Q Why do you think penicillin is no longer as effective as it once was?

The First Synthetic Drugs

Paul Ehrlich, a German physician, was the imaginative thinker who fired the first shot in the chemotherapy revolution. As a medical student, Ehrlich speculated about a “magic bullet” that could hunt down and destroy a pathogen without harming the infected host. He then launched a search for such a bullet. In 1910, after testing hundreds of substances, he found a chemotherapeutic agent called *salvarsan*, an arsenic derivative effective against syphilis. The agent was named salvarsan because it was considered to offer salvation from syphilis and it contained arsenic. Before this discovery, the only known chemical in Europe’s medical arsenal was an extract from the bark of a South American tree, *quinine*, which had been used by Spanish conquistadors to treat malaria.

By the late 1930s, researchers had developed several other synthetic drugs that could destroy microorganisms. Most of these drugs were derivatives of dyes. This came about because the dyes synthesized and manufactured for fabrics were routinely tested for antimicrobial qualities by microbiologists looking for a “magic bullet.” In addition, *sulfonamides* (sulfa drugs) were synthesized at about the same time.

A Fortunate Accident—Antibiotics

In contrast to the sulfa drugs, which were deliberately developed from a series of industrial chemicals, the first antibiotic was discovered by accident. Alexander Fleming, a Scottish physician and bacteriologist, almost tossed out some culture plates that had been contaminated by mold. Fortunately, he took a second look at the curious pattern of growth on the contaminated plates. Around the mold was a clear area where bacterial growth had been inhibited (**Figure 1.5**). Fleming was looking at a mold that

could inhibit the growth of a bacterium. The mold was later identified as *Penicillium notatum* (pen-i-sil’lē-um nō-tā’tum), later renamed *Penicillium chrysogenum* (krī-so’jen-um), and in 1928 Fleming named the mold’s active inhibitor *penicillin*. Thus, penicillin is an antibiotic produced by a fungus. The enormous usefulness of penicillin was not apparent until the 1940s, when it was finally tested clinically and mass produced.

Since these early discoveries, thousands of other antibiotics have been discovered. Unfortunately, antibiotics and other chemotherapeutic drugs are not without problems. Many antimicrobial chemicals are too toxic to humans for practical use; they kill the pathogenic microbes, but they also damage the infected host. For reasons we will discuss later, toxicity to humans is a particular problem in the development of drugs for treating viral diseases. Viral growth depends on life processes of normal host cells. Thus, there are very few successful antiviral drugs, because a drug that would interfere with viral reproduction would also likely affect uninfected cells of the body.

Another major problem associated with antimicrobial drugs is the emergence and spread of new strains of microorganisms that are resistant to antibiotics. Over the years, more and more microbes have developed resistance to antibiotics that at one time were very effective against them. Drug resistance results from genetic changes in microbes that enables them to tolerate a certain amount of an antibiotic that would normally inhibit them (see the box in Chapter 26, page 757). For example a microbe might produce chemicals (enzymes) that inactivate antibiotics, or a microbe might undergo changes to its surface that prevent an antibiotic from attaching to it or entering it.

The recent appearance of vancomycin-resistant *Staphylococcus aureus* and *Enterococcus faecalis* (en-te-rō-kok’kus fe-kā’lis) has alarmed health care professionals because it indicates that some previously treatable bacterial infections may soon be impossible to treat with antibiotics.

CHECK YOUR UNDERSTANDING

What was Ehrlich’s “magic bullet”? **1-11**

Modern Developments in Microbiology

The quest to solve drug resistance, identify viruses, and develop vaccines requires sophisticated research techniques and correlated studies that were never dreamed of in the days of Koch and Pasteur.

The groundwork laid during the Golden Age of Microbiology provided the basis for several monumental achievements during the twentieth century (**Table 1.2**). New branches of microbiology were developed, including immunology and virology. Most recently, the development of a set of new methods called recombinant DNA technology has revolutionized research and practical applications in all areas of microbiology.

Bacteriology, Mycology, and Parasitology

Bacteriology, the study of bacteria, began with van Leeuwenhoek’s first examination of tooth scrapings. New pathogenic

TABLE 1.2 Selected Nobel Prizes Awarded for Research in Microbiology

Nobel Laureates	Year of Presentation	Country of Birth	Contribution
Ronald Ross	1902	England	Discovered how malaria is transmitted
Selman A. Waksman	1952	Ukraine	Discovered streptomycin
Hans A. Krebs	1953	Germany	Discovered chemical steps of the Krebs cycle in carbohydrate metabolism
John F. Enders, Thomas H. Weller, and Frederick C. Robbins	1954	United States	Cultured poliovirus in cell cultures
Joshua Lederberg, George Beadle, and Edward Tatum	1958	United States	Described genetic control of biochemical reactions
Frank Macfarlane Burnet and Peter Brian Medawar	1960	Australia Great Britain	Discovered acquired immune tolerance
César Milstein, Georges J. F. Köhler, and Niels Kai Jerne	1984	Argentina Germany Denmark	Developed a technique for producing monoclonal antibodies (single pure antibodies)
Susumu Tonegawa	1987	Japan	Described the genetics of antibody production
J. Michael Bishop and Harold E. Varmus	1989	United States	Discovered cancer-causing genes called oncogenes
Joseph E. Murray and E. Donnall Thomas	1990	United States	Performed the first successful organ transplants by using immunosuppressive agents
Edmond H. Fisher and Edwin G. Krebs	1992	United States	Discovered protein kinases, enzymes that regulate cell growth
Richard J. Roberts and Phillip A. Sharp	1993	Great Britain United States	Discovered that a gene can be separated onto different segments of DNA
Kary B. Mullis	1993	United States	Discovered the polymerase chain reaction to amplify (make multiple copies of) DNA
Peter C. Doherty and Rolf M. Zinkernagel	1996	Australia Switzerland	Discovered how cytotoxic T cells recognize virus-infected cells prior to destroying them
Peter Agre and Roderick MacKinnon	2003	United States	Discovered water and ion channels in plasma membranes
Aaron Ciechanover, Avram Hershko, and Irwin Rose	2004	Israel Israel United States	Discovered how cells dispose of unwanted proteins in proteasomes
Barry Marshall and J. Robin Warren	2005	Australia	Discovered that <i>Helicobacter pylori</i> causes peptic ulcers
Andrew Fire and Craig Mello	2006	United States	Discovered RNA interference (RNAi), or gene silencing, by double-stranded RNA
Harald zur Hausen	2008	Germany	Discovered that human papilloma viruses cause cervical cancer
Françoise Barré-Sinoussi and Luc Montagnier	2008	France	Discovered human immunodeficiency virus (HIV)
Venkatraman Ramakrishnan, Thomas A. Steitz, and Ada E. Yonath	2010	India United States Israel	Detailed study of the structure and function of ribosomes



(a) Rod of Asclepius, symbol of the medical profession.



(b) A parasitic guinea worm (*Dracunculus medinensis*) is removed from the subcutaneous tissue of a patient by winding it onto a stick. This procedure may have been used for the design of the symbol in part (a).

Figure 1.6 Parasitology: the study of protozoa and parasitic worms.

Q How do you think parasitic worms survive and live off a human host?

bacteria are still discovered regularly. Many bacteriologists, like Pasteur, look at the roles of bacteria in food and the environment. One intriguing discovery came in 1997, when Heide Schulz discovered a bacterium large enough to be seen with the unaided eye (0.2 mm wide). This bacterium, named *Thiomargarita namibiensis* (thī'ō-mă-găr-e-tă na'mīb-ē-ën-sis), lives in the mud on the African coast. *Thiomargarita* is unusual because of its size and its ecological niche. The bacterium consumes hydrogen sulfide, which would be toxic to mud-dwelling animals (Figure 11.28, page 327).

Mycology, the study of fungi, includes medical, agricultural, and ecological branches. Recall that Bassi's work leading up to the germ theory of disease focused on a fungal pathogen. Fungal infection rates have been rising during the past decade, accounting for 10% of hospital-acquired infections. Climatic and environmental changes (severe drought) are thought to account for the tenfold increase in *Coccidioides immitis* (kok-sid-ē-oi'dēz im'mi-tis) infections in California. New techniques for diagnosing and treating fungal infections are currently being investigated.

Parasitology is the study of protozoa and parasitic worms. Because many parasitic worms are large enough to be seen with the unaided eye, they have been known for thousands of years. It has been speculated that the medical symbol, the rod of Asclepius, represents the removal of parasitic guinea worms (Figure 1.6). Asclepius was a Greek physician who practiced about 1200 B.C. and was deified as the god of medicine.

The clearing of rain forests has exposed laborers to previously undiscovered parasites. Previously unknown parasitic diseases are also being found in patients whose immune systems have been suppressed by organ transplants, cancer chemotherapy, or AIDS.

Bacteriology, mycology, and parasitology are currently going through a "golden age" of classification. Recent advances in **genomics**, the study of all of an organism's genes, have allowed

scientists to classify bacteria and fungi according to their genetic relationships with other bacteria, fungi, and protozoa. These microorganisms were originally classified according to a limited number of visible characteristics.

Immunology

Immunology, the study of immunity, dates back in Western culture to Jenner's first vaccine in 1796. Since then, knowledge about the immune system has accumulated steadily and expanded rapidly. Vaccines are now available for numerous diseases, including measles, rubella (German measles), mumps, chickenpox, pneumococcal pneumonia, tetanus, tuberculosis, influenza, whooping cough, polio, and hepatitis B. The smallpox vaccine was so effective that the disease has been eliminated. Public health officials estimate that polio will be eradicated within a few years because of the polio vaccine.

A major advance in immunology occurred in 1933, when Rebecca Lancefield proposed that streptococci be classified according to serotypes (variants within a species) based on certain components in the cell walls of the bacteria. Streptococci are responsible for a variety of diseases, such as sore throat (strep throat), streptococcal toxic shock, and septicemia (blood poisoning). Her research permits the rapid identification of specific pathogenic streptococci based on immunological techniques.

In 1960, interferons, substances generated by the body's own immune system, were discovered. Interferons inhibit replication of viruses and have triggered considerable research related to the treatment of viral diseases and cancer. One of today's biggest challenges for immunologists is learning how the immune system might be stimulated to ward off the virus responsible for AIDS, a disease that destroys the immune system.

Virology

The study of viruses, **virology**, originated during the Golden Age of Microbiology. In 1892, Dmitri Iwanowski reported that the organism that caused mosaic disease of tobacco was so small that it passed through filters fine enough to stop all known bacteria. At the time, Iwanowski was not aware that the organism in question was a virus. In 1935, Wendell Stanley demonstrated that the organism, called tobacco mosaic virus (TMV), was fundamentally different from other microbes and so simple and homogeneous that it could be crystallized like a chemical compound. Stanley's work facilitated the study of viral structure and chemistry. Since the development of the electron microscope in the 1940s, microbiologists have been able to observe the structure of viruses in detail, and today much is known about their structure and activity.

Recombinant DNA Technology

Microorganisms can now be genetically modified to manufacture large amounts of human hormones and other urgently needed medical substances. In the late 1960s, Paul Berg showed that fragments of human or animal DNA (genes) that code for important proteins can be attached to bacterial DNA. The resulting hybrid was the

first example of **recombinant DNA**. When recombinant DNA is inserted into bacteria (or other microbes), it can be used to make large quantities of the desired protein. The technology that developed from this technique is called **recombinant DNA technology**. Its origins can be found in two related fields. The first, **microbial genetics**, studies the mechanisms by which microorganisms inherit traits. The second, **molecular biology**, specifically studies how genetic information is carried in molecules of DNA and how DNA directs the synthesis of proteins.

Although molecular biology encompasses all organisms, much of our knowledge of how genes determine specific traits has been revealed through experiments with bacteria. Through the 1930s, all genetic research was based on the study of plant and animal cells. But in the 1940s, scientists turned to unicellular organisms, primarily bacteria, which have several advantages for genetic and biochemical research. For one thing, bacteria are less complex than plants and animals. For another, the life cycles of many bacteria last less than an hour, so scientists can cultivate very large numbers of bacteria for study in a relatively short time.

Once science turned to the study of unicellular life, rapid progress was made in genetics. In 1941, George W. Beadle and Edward L. Tatum demonstrated the relationship between genes and enzymes. DNA was established as the hereditary material in 1944 by Oswald Avery, Colin MacLeod, and Maclyn McCarty. In 1946, Joshua Lederberg and Edward L. Tatum discovered that genetic material could be transferred from one bacterium to another by a process called conjugation. Then, in 1953, James Watson and Francis Crick proposed a model for the structure and replication of DNA. The early 1960s witnessed a further explosion of discoveries relating to the way DNA controls protein synthesis. In 1961, François Jacob and Jacques Monod discovered messenger RNA (ribonucleic acid), a chemical involved in protein synthesis, and later they made the first major discoveries about the regulation of gene function in bacteria. During the same period, scientists were able to break the genetic code and thus understand how the information for protein synthesis in messenger RNA is translated into the amino acid sequence for making proteins.

CHECK YOUR UNDERSTANDING

- ✓ Define *bacteriology, mycology, parasitology, immunology, and virology*. **1-12**
- ✓ Differentiate microbial genetics from molecular biology. **1-13**

Microbes and Human Welfare

LEARNING OBJECTIVES

- 1-14** List at least four beneficial activities of microorganisms.
- 1-15** Name two examples of biotechnology that use recombinant DNA technology and two examples that do not.

As mentioned earlier, only a minority of all microorganisms are pathogenic. Microbes that cause food spoilage, such as soft spots on fruits and vegetables, decomposition of meats, and rancidity

of fats and oils, are also a minority. The vast majority of microbes benefit humans, other animals, and plants in many ways. For example, microbes produce methane and ethanol that can be used as alternative fuels to generate electricity and power vehicles. Biotechnology companies are using bacterial enzymes to break down plant cellulose so that yeast can metabolize the resulting simple sugars and produce ethanol. The following sections outline some of these beneficial activities. In later chapters, we will discuss these activities in greater detail.

Recycling Vital Elements

Discoveries made by two microbiologists in the 1880s have formed the basis for today's understanding of the biogeochemical cycles that support life on Earth. Martinus Beijerinck and Sergei Winogradsky were the first to show how bacteria help recycle vital elements between the soil and the atmosphere. **Microbial ecology**, the study of the relationship between microorganisms and their environment, originated with the work of these scientists. Today, microbial ecology has branched out and includes the study of how microbial populations interact with plants and animals in various environments. Among the concerns of microbial ecologists are water pollution and toxic chemicals in the environment.

The chemical elements carbon, nitrogen, oxygen, sulfur, and phosphorus are essential for life and abundant, but not necessarily in forms that organisms can use. Microorganisms are primarily responsible for converting these elements into forms that plants and animals can use. Microorganisms, primarily bacteria and fungi, return carbon dioxide to the atmosphere when they decompose organic wastes and dead plants and animals. Algae, cyanobacteria, and higher plants use the carbon dioxide during photosynthesis to produce carbohydrates for animals, fungi, and bacteria. Nitrogen is abundant in the atmosphere but in that form is not usable by plants and animals. Only bacteria can naturally convert atmospheric nitrogen to a form available to plants and animals.

Sewage Treatment: Using Microbes to Recycle Water

Our society's growing awareness of the need to preserve the environment has made people more conscious of the responsibility to recycle precious water and prevent the pollution of rivers and oceans. One major pollutant is sewage, which consists of human excrement, waste water, industrial wastes, and surface runoff. Sewage is about 99.9% water, with a few hundredths of 1% suspended solids. The remainder is a variety of dissolved materials.

Sewage treatment plants remove the undesirable materials and harmful microorganisms. Treatments combine various physical processes with the action of beneficial microbes. Large solids such as paper, wood, glass, gravel, and plastic are removed from sewage; left behind are liquid and organic materials that bacteria convert into such by-products as carbon dioxide, nitrates, phosphates, sulfates, ammonia, hydrogen sulfide, and methane. (We will discuss sewage treatment in detail in Chapter 27.)

Bioremediation: Using Microbes to Clean Up Pollutants

In 1988, scientists began using microbes to clean up pollutants and toxic wastes produced by various industrial processes. For example, some bacteria can actually use pollutants as energy sources; others produce enzymes that break down toxins into less harmful substances. By using bacteria in these ways—a process known as **bioremediation**—toxins can be removed from underground wells, chemical spills, toxic waste sites, and oil spills, such as the massive oil spill from an offshore drilling rig in the Gulf of Mexico on April 20, 2010 (see also the box in Chapter 2, page 32). In addition, bacterial enzymes are used in drain cleaners to remove clogs without adding harmful chemicals to the environment. In some cases, microorganisms indigenous to the environment are used; in others, genetically modified microbes are used. Among the most commonly used microbes are certain species of bacteria of the genera *Pseudomonas* (sū-dō-mō'nas) and *Bacillus* (bä-sil'lus). *Bacillus* enzymes are also used in household detergents to remove spots from clothing.

Insect Pest Control by Microorganisms

Besides spreading diseases, insects can cause devastating crop damage. Insect pest control is therefore important for both agriculture and the prevention of human disease.

The bacterium *Bacillus thuringiensis* (thür-in-jē-en'sis) has been used extensively in the United States to control such pests as alfalfa caterpillars, bollworms, corn borers, cabbageworms, tobacco budworms, and fruit tree leaf rollers. It is incorporated into a dusting powder that is applied to the crops these insects eat. The bacteria produce protein crystals that are toxic to the digestive systems of the insects. The toxin gene also has been inserted into some plants to make them insect resistant.

By using microbial rather than chemical insect control, farmers can avoid harming the environment. Many chemical insecticides, such as DDT, remain in the soil as toxic pollutants and are eventually incorporated into the food chain.

Modern Biotechnology and Recombinant DNA Technology

Earlier, we touched on the commercial use of microorganisms to produce some common foods and chemicals. Such practical applications of microbiology are called **biotechnology**. Although biotechnology has been used in some form for centuries, techniques have become much more sophisticated in the past few decades. In the last several years, biotechnology has undergone a revolution through the advent of recombinant DNA technology to expand the potential of bacteria, viruses, and yeast cells and other fungi as miniature biochemical factories. Cultured plant and animal cells, as well as intact plants and animals, are also used as recombinant cells and organisms.

The applications of recombinant DNA technology are increasing with each passing year. Recombinant DNA techniques have been used thus far to produce a number of natural proteins, vaccines, and enzymes. Such substances have great potential for medical use; some of them are described in Table 9.1 on page 248.

A very exciting and important outcome of recombinant DNA techniques is **gene therapy**—inserting a missing gene or replacing a defective one in human cells. This technique uses a harmless virus to carry the missing or new gene into certain host cells, where the gene is picked up and inserted into the appropriate chromosome. Since 1990, gene therapy has been used to treat patients with adenosine deaminase (ADA) deficiency, a cause of severe combined immunodeficiency disease (SCID), in which cells of the immune system are inactive or missing; Duchenne's muscular dystrophy, a muscle-destroying disease; cystic fibrosis, a disease of the secreting portions of the respiratory passages, pancreas, salivary glands, and sweat glands; and LDL-receptor deficiency, a condition in which low-density lipoprotein (LDL) receptors are defective and LDL cannot enter cells. The LDL remains in the blood in high concentrations and increases the risk of atherosclerosis and coronary artery disease because it leads to fatty plaque formation in blood vessels. Results are still being evaluated. Other genetic diseases may also be treatable by gene therapy in the future, including hemophilia, an inability of the blood to clot normally; diabetes, elevated blood sugar levels; sickle cell disease, an abnormal kind of hemoglobin; and one type of hypercholesterolemia, high blood cholesterol.

Beyond medical applications, recombinant DNA techniques have also been applied to agriculture. For example, genetically altered strains of bacteria have been developed to protect fruit against frost damage, and bacteria are being modified to control insects that damage crops. Recombinant DNA has also been used to improve the appearance, flavor, and shelf life of fruits and vegetables. Potential agricultural uses of recombinant DNA include drought resistance, resistance to insects and microbial diseases, and increased temperature tolerance in crops.

CHECK YOUR UNDERSTANDING

- ✓ Name two beneficial uses of bacteria. **1-14**
- ✓ Differentiate biotechnology from recombinant DNA technology. **1-15**

Microbes and Human Disease

LEARNING OBJECTIVES

- 1-16** Define *normal microbiota* and *resistance*.
- 1-17** Define *biofilm*.
- 1-18** Define *emerging infectious disease*.

Normal Microbiota

We all live from birth until death in a world filled with microbes, and we all have a variety of microorganisms on and inside our

bodies. These microorganisms make up our **normal microbiota**, or *flora** (Figure 1.7). The normal microbiota not only do us no harm, but also in some cases can actually benefit us. For example, some normal microbiota protect us against disease by preventing the overgrowth of harmful microbes, and others produce useful substances such as vitamin K and some B vitamins. Unfortunately, under some circumstances normal microbiota can make us sick or infect people we contact. For instance, when some normal microbiota leave their habitat, they can cause disease.

When is a microbe a welcome part of a healthy human, and when is it a harbinger of disease? The distinction between health and disease is in large part a balance between the natural defenses of the body and the disease-producing properties of microorganisms. Whether our bodies overcome the offensive tactics of a particular microbe depends on our **resistance**—the ability to ward off diseases. Important resistance is provided by the barrier of the skin, mucous membranes, cilia, stomach acid, and antimicrobial chemicals such as interferons. Microbes can be destroyed by white blood cells, by the inflammatory response, by fever, and by specific responses of our immune system. Sometimes, when our natural defenses are not strong enough to overcome an invader, they have to be supplemented by antibiotics or other drugs.

Clinical Case

Staph is the common name for *Staphylococcus aureus* bacteria, which are carried on the skin of about 30% of the human population. Although Andrea is diligent about taking her antibiotic as prescribed, she doesn't seem to be improving. After 3 days, the lesion on her wrist is even larger than before and is now draining yellow pus. Andrea also develops a fever. Her mother insists that she call her doctor to tell him about the latest developments.

Why does Andrea's infection persist after treatment?

2 17 19 20 21

Biofilms

In nature, microorganisms may exist as single cells that float or swim independently in a liquid, or they may attach to each other and/or some usually solid surface. This latter mode of behavior is called a **biofilm**, a complex aggregation of microbes. The slime covering a rock in a lake is a biofilm. Use your tongue to feel the biofilm on your teeth. Biofilms can be beneficial. They protect your mucous membranes from harmful microbes, and biofilms in lakes are an important food for aquatic animals. Biofilms can also be harmful. They can clog water pipes, and on medical implants

* At one time, bacteria and fungi were thought to be plants, and thus the term *flora* was used.



Figure 1.7 Several types of bacteria found as part of the normal microbiota on the surface of the human tongue.

Q How do we benefit from the production of vitamin K by microbes?

such as joint prostheses and catheters (Figure 1.8), they can cause such infections as endocarditis (inflammation of the heart). Bacteria in biofilms are often resistant to antibiotics because the biofilm offers a protective barrier. See the box in Chapter 3 on page 56. Biofilms will be discussed in Chapter 6.

Infectious Diseases

An **infectious disease** is a disease in which pathogens invade a susceptible host, such as a human or an animal. In the process, the pathogen carries out at least part of its life cycle inside the host, and disease frequently results. By the end of World War II, many people believed that infectious diseases were under control. They thought malaria would be eradicated through the use of the insecticide DDT to kill mosquitoes, that a vaccine would prevent diphtheria, and that improved sanitation measures would help prevent cholera transmission. Malaria is far from eliminated. Since 1986, local outbreaks have been identified in New Jersey, California, Florida, New York, and Texas, and the disease infects 300 million people worldwide. In 1994, diphtheria appeared in the United States, brought by travelers from the newly independent states of the former Soviet Union, which were experiencing a massive diphtheria epidemic. The epidemic was brought under control in 1998. Cholera outbreaks still occur in less-developed parts of the world.

Emerging Infectious Diseases

These recent outbreaks point to the fact that infectious diseases are not disappearing, but rather seem to be reemerging and increasing. In addition, a number of new diseases—**emerging infectious diseases (EIDs)**—have cropped up in recent years. These are diseases that are new or changing and are increasing

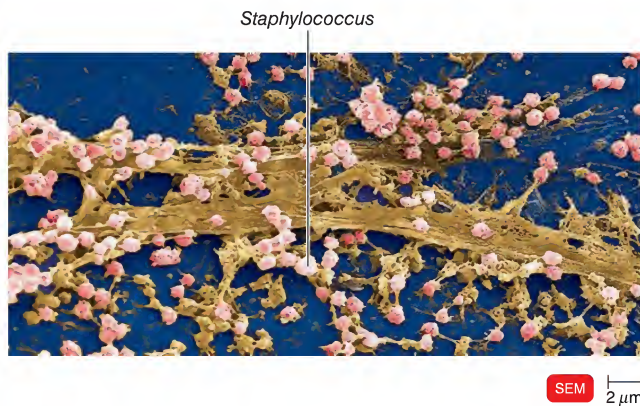


Figure 1.8 Biofilm on a catheter. *Staphylococcus* bacteria stick to solid surfaces, forming a slimy layer. Bacteria that break away from this biofilm can cause infections.

Q How does a biofilm's protective barrier make it resistant to antibiotics?

or have the potential to increase in incidence in the near future. Some of the factors that have contributed to the development of EIDs are evolutionary changes in existing organisms (e.g., *Vibrio cholerae*; vib'rē-ō kol'-er-ī); the spread of known diseases to new geographic regions or populations by modern transportation (e.g., West Nile virus); and increased human exposure to new, unusual infectious agents in areas that are undergoing ecologic changes such as deforestation and construction (e.g., Venezuelan hemorrhagic virus). EIDs also develop as a result of antimicrobial resistance (e.g., vancomycin-resistant *S. aureus*). An increasing number of incidents in recent years highlights the extent of the problem.

H1N1 influenza (flu), also known as *swine flu*, is a type of influenza caused by a new virus called *influenza H1N1*. H1N1 was first detected in the United States in April 2009. In June 2009, the World Health Organization declared H1N1 flu to be a *global pandemic disease* (a disease that affects large numbers of individuals in a short period of time and occurs worldwide).

Avian influenza A (H5N1), or bird flu, caught the attention of the public in 2003, when it killed millions of poultry and 24 people in eight countries in southeast Asia. Avian influenza viruses occur in birds worldwide. Certain wild birds, particularly waterfowl, do not get sick but carry the virus in their intestines and shed it in saliva, nasal secretions, and feces. Most often, the wild birds spread influenza to domesticated birds, in which the virus causes death.

Influenza A viruses are found in many different animals, including ducks, chickens, pigs, whales, horses, and seals. Normally, each subtype of influenza A virus is specific to certain species. However, influenza A viruses normally seen in one species sometimes can cross over and cause illness in another species, and all subtypes of influenza A virus can infect pigs. Although

it is unusual for people to get influenza infections directly from animals, sporadic human infections and outbreaks caused by certain avian influenza A viruses and pig influenza viruses have been reported. As of 2008, avian influenza had sickened 242 people, and about half of them died. Fortunately, the virus has not yet evolved to be transmitted successfully among humans.

Human infections with avian influenza viruses detected since 1997 have not resulted in sustained human-to-human transmission. However, because influenza viruses have the potential to change and gain the ability to spread easily between people, monitoring for human infection and person-to-person transmission is important (see the box in Chapter 13 on page 374). The U.S. Food and Drug Administration (FDA) approved a human vaccine against the avian influenza virus in April 2007.

Antibiotics are critical in treating bacterial infections. However, years of overuse and misuse of these drugs have created environments in which antibiotic-resistant bacteria thrive. Random mutations in bacterial genes can make a bacterium resistant to an antibiotic. In the presence of that antibiotic, this bacterium has an advantage over other, susceptible bacteria and is able to proliferate. Antibiotic-resistant bacteria have become a global health crisis.

Staphylococcus aureus causes a wide range of human infections from pimples and boils to pneumonia, food poisoning, and surgical wound infections, and it is a significant cause of hospital-associated infections. After penicillin's initial success in treating *S. aureus* infection, penicillin-resistant *S. aureus* became a major threat in hospitals in the 1950s, requiring the use of methicillin. In the 1980s, **methicillin-resistant *S. aureus***, called **MRSA**, emerged and became endemic in many hospitals, leading to increasing use of vancomycin. In the late 1990s, *S. aureus* infections that were less sensitive to vancomycin (**vancomycin-intermediate *S. aureus***, or **VISA**) were reported. In 2002, an infection caused by **vancomycin-resistant *S. aureus*** (**VRSA**) in a patient in the United States was reported.

In March 2010, the World Health Organization (WHO) reported that in some parts of the world (such as northwestern Russia) about 28% of all individuals with tuberculosis (TB) had the multidrug-resistant form of the disease (MDR-TB). Multidrug-resistant TB is caused by bacteria that are resistant to at least the antibiotics isoniazid and rifampicin, the most effective drugs against tuberculosis.

The antibacterial substances added to various household cleaning products are similar to antibiotics in many ways. When used correctly, they inhibit bacterial growth. However, wiping every household surface with these antibacterial agents creates an environment in which the resistant bacteria survive. Unfortunately, when you really need to disinfect your homes and hands—for example, when a family member comes home from a hospital and is still vulnerable to infection—you may encounter mainly resistant bacteria.

Routine housecleaning and handwashing are necessary, but standard soaps and detergents (without added antibacterials) are fine for these tasks. In addition, quickly evaporating chemicals, such as chlorine bleach, alcohol, ammonia, and hydrogen peroxide, remove potentially pathogenic bacteria but do not leave residues that encourage the growth of resistant bacteria.

Clinical Case

The *S. aureus* bacterium responsible for Andrea's infection is resistant to the β -lactam antibiotic prescribed by Andrea's doctor. Concerned about what his patient is telling him, Andrea's doctor calls the local hospital to let them know he is sending a patient over. In the emergency department, a nurse swabs Andrea's wound and sends it to the hospital lab for culturing. The culture shows that Andrea's infection is caused by methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA produces β -lactamase, an enzyme that destroys β -lactam antibiotics. The attending physician surgically drains the pus from the sore on Andrea's wrist.

How does antibiotic resistance develop?

2 17 **19** 20 21

West Nile encephalitis (WNE) is inflammation of the brain caused by West Nile virus (see Chapter 8). WNE was first diagnosed in the West Nile region of Uganda in 1937. In 1999 the virus made its first North American appearance in humans in New York City. In 2007, West Nile virus infected over 3600 people in 43 states. West Nile virus is now established in non-migratory birds in 48 states. The virus, which is carried by birds, is transmitted between birds—and to horses and humans—by mosquitoes. West Nile virus may have arrived in the United States in an infected traveler or in migratory birds.

In 1996, countries worldwide were refusing to import beef from the United Kingdom, where hundreds of thousands of cattle born after 1988 had to be killed because of an epidemic of **bovine spongiform encephalopathy** (en-sef-a-lop'a-thē), also called **BSE** or **mad cow disease**. BSE first came to the attention of microbiologists in 1986 as one of a handful of diseases caused by an infectious protein called a *prion*. Studies suggest that the source of disease was cattle feed prepared from sheep infected with their own version of the disease. Cattle are herbivores (plant eaters), but adding protein to their feed improves their growth and health. **Creutzfeldt-Jakob disease** (kroits'felt yä'kôb), or **CJD**, is a human disease also caused by a prion. The incidence of CJD in the United Kingdom is similar to the incidence in other countries. However, by 2005 the United Kingdom reported 154 human cases of CJD caused by a new variant related to the bovine disease (see Chapter 22).

Escherichia coli is a normal inhabitant of the large intestine of vertebrates, including humans, and its presence is beneficial

because it helps produce certain vitamins and breaks down otherwise undigestible foodstuffs (see Chapter 25). However, a strain called *E. coli* O157:H7 causes bloody diarrhea when it grows in the intestines. This strain was first recognized in 1982 and since then has emerged as a public health problem. It is now one of the leading causes of diarrhea worldwide. In 1996, some 9000 people in Japan became ill, and 7 died, as a result of infection by *E. coli* O157:H7. The recent outbreaks of *E. coli* O157:H7 in the United States, associated with contamination of undercooked meat and unpasteurized beverages, have led public health officials to call for the development of new methods of testing for bacteria in food.

In 1995, infections of so-called **flesh-eating bacteria** were reported on the front pages of major newspapers. The bacteria are more correctly named invasive group A *Streptococcus* (strep-tō-kok'kus), or IGAS. Rates of IGAS in the United States, Scandinavia, England, and Wales have been increasing.

In 1995, a hospital laboratory technician in Democratic Republic of Congo (DROC) who had fever and bloody diarrhea underwent surgery for a suspected perforated bowel. Afterward he started hemorrhaging, and his blood began clotting in his blood vessels. A few days later, health care workers in the hospital where he was staying developed similar symptoms. One of them was transferred to a hospital in a different city; personnel in the second hospital who cared for this patient also developed symptoms. By the time the epidemic was over, 315 people had contracted **Ebola hemorrhagic fever** (hem-ô-raj'ik), or **EHF**, and over 75% of them died. The epidemic was controlled when microbiologists instituted training on the use of protective equipment and educational measures in the community. Close personal contact with infectious blood or other body fluids or tissue (see Chapter 23) leads to human-to-human transmission.

Microbiologists first isolated Ebola viruses from humans during earlier outbreaks in DROC in 1976. (The virus is named after Congo's Ebola River.) In 2008, an Ebola virus outbreak occurred in Uganda with 149 cases. In 1989 and 1996, outbreaks among monkeys imported into the United States from the Philippines were caused by another Ebola virus but were not associated with human disease.

Recorded cases of **Marburg virus**, another hemorrhagic fever virus, are rare. The first cases were laboratory workers in Europe who handled African green monkeys from Uganda. Four outbreaks were identified in Africa between 1975 and 1998, involving 2 to 154 people with 56% mortality. In 2004, an outbreak killed 227 people. Microbiologists have been studying many animals but have not yet discovered the natural reservoir (source) of EHF and Marburg viruses.

In 1993, an outbreak of **cryptosporidiosis** (krip-tō-spō-rid-ē-ō'sis) transmitted through the public water supply in Milwaukee, Wisconsin, resulted in diarrheal illness in an estimated 403,000 persons. The microorganism responsible for this outbreak was the protozoan *Cryptosporidium* (krip-tō-spō-ri'dē-um). First

reported as a cause of human disease in 1976, it is responsible for up to 30% of the diarrheal illness in developing countries. In the United States, transmission has occurred via drinking water, swimming pools, and contaminated hospital supplies.

AIDS (acquired immunodeficiency syndrome) first came to public attention in 1981 with reports from Los Angeles that a few young homosexual men had died of a previously rare type of pneumonia known as *Pneumocystis* (nü-mō-sis'tis) pneumonia. These men had experienced a severe weakening of the immune system, which normally fights infectious diseases. Soon these cases were correlated with an unusual number of occurrences of a rare form of cancer, Kaposi's sarcoma, among young homosexual men. Similar increases in such rare diseases were found among hemophiliacs and intravenous drug users.

Researchers quickly discovered that the cause of AIDS was a previously unknown virus (see Figure 1.1e). The virus, now called **human immunodeficiency virus (HIV)**, destroys CD4⁺ T cells, one type of white blood cell important to immune system defenses. Sickness and death result from microorganisms or cancerous cells that might otherwise have been defeated by the body's natural defenses. So far, the disease has been inevitably fatal once symptoms develop.

By studying disease patterns, medical researchers found that HIV could be spread through sexual intercourse, by contaminated needles, from infected mothers to their newborns via breast milk, and by blood transfusions—in short, by the transmission of body fluids from one person to another. Since

1985, blood used for transfusions has been carefully checked for the presence of HIV, and it is now quite unlikely that the virus can be spread by this means.

By the end of 2010, over 1 million people in the United States are living with AIDS. Over 50,000 Americans become infected and 18,000 die each year. As of 2010, health officials estimated that 1.3 million Americans have HIV infection. In 2009, the World Health Organization (WHO) estimated that over 33 million people worldwide are living with HIV/AIDS and that 7500 new infections occur every day.

Since 1994, new treatments have extended the life span of people with AIDS; however, approximately 40,000 new cases occur annually in the United States. The majority of individuals with AIDS are in the sexually active age group. Because heterosexual partners of AIDS sufferers are at high risk of infection, public health officials are concerned that even more women and minorities will contract AIDS. In 1997, HIV diagnoses began increasing among women and minorities. Among the AIDS cases reported in 2009, 26% were women, and 49% were African American.

In the months and years to come, scientists will continue to apply microbiological techniques to help them learn more about the structure of the deadly HIV, how it is transmitted, how it grows in cells and causes disease, how drugs can be directed against it, and whether an effective vaccine can be developed. Public health officials have also focused on prevention through education.

AIDS poses one of this century's most formidable health threats, but it is not the first serious epidemic of a sexually transmitted disease. Syphilis was also once a fatal epidemic disease. As recently as 1941, syphilis caused an estimated 14,000 deaths per year in the United States. With few drugs available for treatment and no vaccines to prevent it, efforts to control the disease focused mainly on altering sexual behavior and on the use of condoms. The eventual development of drugs to treat syphilis contributed significantly to preventing the spread of the disease. According to the Centers for Disease Control and Prevention (CDC), reported cases of syphilis dropped from a record high of 575,000 in 1943 to an all-time low of 5979 cases in 2004. Since then, however, the number of cases has been increasing.

Just as microbiological techniques helped researchers in the fight against syphilis and smallpox, they will help scientists discover the causes of new emerging infectious diseases in the twenty-first century. Undoubtedly there will be new diseases. Ebola virus and *Influenzavirus* are examples of viruses that may be changing their abilities to infect different host species. Emerging infectious diseases will be discussed further in Chapter 14 on page 417.

Infectious diseases may reemerge because of antibiotic resistance (see the box in Chapter 26 on page 757) and through the use of microorganisms as weapons. (See the box in Chapter 23 on page 651.) The breakdown of public health measures for previously controlled infections has resulted in unexpected cases of tuberculosis, whooping cough, and diphtheria (see Chapter 24).

Clinical Case

Mutations develop randomly in bacteria: some mutations are lethal, some have no effect, and some may be beneficial. Once these mutations develop, the offspring of the mutated parent cells also carry the same mutation. Because they have an advantage in the presence of the antibiotic, bacteria that are resistant to antibiotics soon outnumber those that are susceptible to antibiotic therapy. The widespread use of antibiotics selectively allows the resistant bacteria to grow, whereas the susceptible bacteria are killed. Eventually, almost the entire population of bacteria is resistant to the antibiotic.

The emergency department physician prescribes a different antibiotic, vancomycin, which will kill the MRSA in Andrea's wrist. She also explains to Andrea what MRSA is and why it's important they find out where Andrea acquired the potentially lethal bacteria.

What can the emergency department physician tell Andrea about MRSA?

2 17 19 **20** 21

CHECK YOUR UNDERSTANDING

- ✓ Differentiate normal microbiota and infectious disease. **1-16**
- ✓ Why are biofilms important? **1-17**
- ✓ What factors contribute to the emergence of an infectious disease? **1-18**

* * *

The diseases we have mentioned are caused by viruses, bacteria, protozoa, and prions—types of microorganisms. This book introduces you to the enormous variety of microscopic organisms. It shows you how microbiologists use specific techniques and procedures to study the microbes that cause such diseases as AIDS and diarrhea—and diseases that have yet to be discovered. You will also learn how the body responds to microbial infection and how certain drugs combat microbial diseases. Finally, you will learn about the many beneficial roles that microbes play in the world around us.

Clinical Case Resolved

The first MRSA was health care–associated MRSA (HA-MRSA), transmitted between staff and patients in health care settings. In the 1990s, infections by a genetically different strain, community-associated MRSA

(CA-MRSA), emerged as a major cause of skin disease in the United States. CA-MRSA enters skin abrasions from environmental surfaces or other people. Andrea has never been hospitalized before now, so they are able to rule out the hospital as the source of infection. Her college courses are all online, so she didn't contract MRSA at the university, either. The local health department sends someone to her family home to swab for the bacteria there.

MRSA is isolated from Andrea's living room sofa, but how did it get there? After speaking with the family, the representative from the health department, knowing that clusters of CA-MRSA infections have been seen among athletes suggests swabbing the mats used by the gymnasts at the school Andrea's sister attends. The cultures come back positive for MRSA. Andrea's sister, although not infected, transferred the bacteria from her skin to the sofa, where Andrea laid her arm. (A person can carry MRSA on the skin without becoming infected.) The bacteria entered through a scratch on Andrea's wrist.

2 17 19 20 **21****Study Outline****MasteringMICROBIOLOGY™**

Test your understanding with quizzes, microbe review, and a chapter post-test at www.masteringmicrobiology.com.

Microbes in Our Lives (p. 2)

1. Living things too small to be seen with the unaided eye are called microorganisms.
2. Microorganisms are important in maintaining Earth's ecological balance.
3. Some microorganisms live in humans and other animals and are needed to maintain good health.
4. Some microorganisms are used to produce foods and chemicals.
5. Some microorganisms cause disease.

Naming and Classifying Microorganisms (pp. 2–6)**Nomenclature** (p. 3)

1. In a nomenclature system designed by Carolus Linnaeus (1735), each living organism is assigned two names.
2. The two names consist of a genus and a specific epithet, both of which are underlined or italicized.

Types of Microorganisms (pp. 3–6)

3. Bacteria are unicellular organisms. Because they have no nucleus, the cells are described as prokaryotic.

4. The three major basic shapes of bacteria are bacillus, coccus, and spiral.
5. Most bacteria have a peptidoglycan cell wall; they divide by binary fission, and they may possess flagella.
6. Bacteria can use a wide range of chemical substances for their nutrition.
7. Archaea consist of prokaryotic cells; they lack peptidoglycan in their cell walls.
8. Archaea include methanogens, extreme halophiles, and extreme thermophiles.
9. Fungi (mushrooms, molds, and yeasts) have eukaryotic cells (cells with a true nucleus). Most fungi are multicellular.
10. Fungi obtain nutrients by absorbing organic material from their environment.
11. Protozoa are unicellular eukaryotes.
12. Protozoa obtain nourishment by absorption or ingestion through specialized structures.
13. Algae are unicellular or multicellular eukaryotes that obtain nourishment by photosynthesis.
14. Algae produce oxygen and carbohydrates that are used by other organisms.
15. Viruses are noncellular entities that are parasites of cells.
16. Viruses consist of a nucleic acid core (DNA or RNA) surrounded by a protein coat. An envelope may surround the coat.
17. The principal groups of multicellular animal parasites are flatworms and roundworms, collectively called helminths.
18. The microscopic stages in the life cycle of helminths are identified by traditional microbiological procedures.

Classification of Microorganisms (p. 6)

19. All organisms are classified into Bacteria, Archaea, and Eukarya. Eukarya include protists, fungi, plants, and animals.

A Brief History of Microbiology (pp. 6–15)**The First Observations** (p. 6)

1. Robert Hooke observed that cork was composed of “little boxes”; he introduced the term *cell* (1665).
2. Hooke’s observations laid the groundwork for development of the cell theory, the concept that all living things are composed of cells.
3. Anton van Leeuwenhoek, using a simple microscope, was the first to observe microorganisms (1673).

The Debate over Spontaneous Generation (pp. 6–8)

4. Until the mid-1880s, many people believed in spontaneous generation, the idea that living organisms could arise from nonliving matter.
5. Francesco Redi demonstrated that maggots appear on decaying meat only when flies are able to lay eggs on the meat (1668).
6. John Needham claimed that microorganisms could arise spontaneously from heated nutrient broth (1745).
7. Lazzaro Spallanzani repeated Needham’s experiments and suggested that Needham’s results were due to microorganisms in the air entering his broth (1765).
8. Rudolf Virchow introduced the concept of biogenesis: living cells can arise only from preexisting cells (1858).
9. Louis Pasteur demonstrated that microorganisms are in the air everywhere and offered proof of biogenesis (1861).
10. Pasteur’s discoveries led to the development of aseptic techniques used in laboratory and medical procedures to prevent contamination by microorganisms.

The Golden Age of Microbiology (pp. 8–11)

11. The science of microbiology advanced rapidly between 1857 and 1914.
12. Pasteur found that yeasts ferment sugars to alcohol and that bacteria can oxidize the alcohol to acetic acid.
13. A heating process called pasteurization is used to kill bacteria in some alcoholic beverages and milk.
14. Agostino Bassi (1835) and Pasteur (1865) showed a causal relationship between microorganisms and disease.
15. Joseph Lister introduced the use of a disinfectant to clean surgical wounds in order to control infections in humans (1860s).
16. Robert Koch proved that microorganisms cause disease. He used a sequence of procedures, now called Koch’s postulates (1876), that are used today to prove that a particular microorganism causes a particular disease.
17. In a vaccination, immunity (resistance to a particular disease) is conferred by inoculation with a vaccine.
18. In 1798, Edward Jenner demonstrated that inoculation with cowpox material provides humans with immunity to smallpox.
19. About 1880, Pasteur discovered that avirulent bacteria could be used as a vaccine for fowl cholera; he coined the word *vaccine*.
20. Modern vaccines are prepared from living avirulent microorganisms or killed pathogens, from isolated components of pathogens, and by recombinant DNA techniques.

The Birth of Modern Chemotherapy:**Dreams of a “Magic Bullet”** (pp. 11–12)

21. Chemotherapy is the chemical treatment of a disease.

22. Two types of chemotherapeutic agents are synthetic drugs (chemically prepared in the laboratory) and antibiotics (substances produced naturally by bacteria and fungi to inhibit the growth of other microorganisms).
23. Paul Ehrlich introduced an arsenic-containing chemical called salvarsan to treat syphilis (1910).
24. Alexander Fleming observed that the *Penicillium* fungus inhibited the growth of a bacterial culture. He named the active ingredient penicillin (1928).
25. Penicillin has been used clinically as an antibiotic since the 1940s.
26. Researchers are tackling the problem of drug-resistant microbes.

Modern Developments in Microbiology (pp. 12–15)

27. Bacteriology is the study of bacteria, mycology is the study of fungi, and parasitology is the study of parasitic protozoa and worms.
28. Microbiologists are using genomics, the study of all of an organism’s genes, to classify bacteria, fungi, and protozoa.
29. The study of AIDS, analysis of the action of interferons, and the development of new vaccines are among the current research interests in immunology.
30. New techniques in molecular biology and electron microscopy have provided tools for advancing our knowledge of virology.
31. The development of recombinant DNA technology has helped advance all areas of microbiology.

Microbes and Human Welfare (pp. 15–16)

1. Microorganisms degrade dead plants and animals and recycle chemical elements to be used by living plants and animals.
2. Bacteria are used to decompose organic matter in sewage.
3. Bioremediation processes use bacteria to clean up toxic wastes.
4. Bacteria that cause diseases in insects are being used as biological controls of insect pests. Biological controls are specific for the pest and do not harm the environment.
5. Using microbes to make products such as foods and chemicals is called biotechnology.
6. Using recombinant DNA, bacteria can produce important substances such as proteins, vaccines, and enzymes.
7. In gene therapy, viruses are used to carry replacements for defective or missing genes into human cells.
8. Genetically modified bacteria are used in agriculture to protect plants from frost and insects and to improve the shelf life of produce.

Microbes and Human Disease (pp. 16–21)

1. Everyone has microorganisms in and on the body; these make up the normal microbiota, or flora.
2. The disease-producing properties of a species of microbe and the host’s resistance are important factors in determining whether a person will contract a disease.
3. Bacterial communities that form slimy layers on surfaces are called biofilms.
4. An infectious disease is one in which pathogens invade a susceptible host.
5. An emerging infectious disease (EID) is a new or changing disease showing an increase in incidence in the recent past or a potential to increase in the near future.

Study Questions

Answers to the Review and Multiple Choice questions can be found by turning to the Answers tab at the back of the textbook.

Review

- How did the idea of spontaneous generation come about?
- Briefly state the role microorganisms play in each of the following:
 - biological control of pests
 - recycling of elements
 - normal microbiota
 - sewage treatment
 - human insulin production
 - vaccine production
 - biofilms
- Into which field of microbiology would the following scientists best fit?

Researcher Who	Field
_____ a. Studies biodegradation of toxic wastes	1. Biotechnology
_____ b. Studies the causative agent of Ebola hemorrhagic fever	2. Immunology
_____ c. Studies the production of human proteins by bacteria	3. Microbial ecology
_____ d. Studies the symptoms of AIDS	4. Microbial genetics
_____ e. Studies the production of toxin by <i>E. coli</i>	5. Microbial physiology
_____ f. Studies the life cycle of <i>Cryptosporidium</i>	6. Molecular biology
_____ g. Develops gene therapy for a disease	7. Mycology
_____ h. Studies the fungus <i>Candida albicans</i>	8. Virology

- Match the microorganisms in column A to their descriptions in column B.

Column A	Column B
_____ a. Archaea	1. Not composed of cells
_____ b. Algae	2. Cell wall made of chitin
_____ c. Bacteria	3. Cell wall made of peptidoglycan
_____ d. Fungi	4. Cell wall made of cellulose; photosynthetic
_____ e. Helminths	5. Unicellular, complex cell structure lacking a cell wall
_____ f. Protozoa	6. Multicellular animals
_____ g. Viruses	7. Prokaryote without peptidoglycan cell wall

- Match the people in column A to their contribution toward the advancement of microbiology, in column B.

Column A	Column B
_____ a. Avery, MacLeod, and McCarty	1. Developed vaccine against smallpox
_____ b. Beadle and Tatum	2. Discovered how DNA controls protein synthesis in a cell
_____ c. Berg	3. Discovered penicillin

- | | |
|------------------------------|---|
| _____ d. Ehrlich | 4. Discovered that DNA can be transferred from one bacterium to another |
| _____ e. Fleming | 5. Disproved spontaneous generation |
| _____ f. Hooke | 6. First to characterize a virus |
| _____ g. Iwanowski | 7. First to use disinfectants in surgical procedures |
| _____ h. Jacob and Monod | 8. First to observe bacteria |
| _____ i. Jenner | 9. First to observe cells in plant material and name them |
| _____ j. Koch | 10. Observed that viruses are filterable |
| _____ k. Lancefield | 11. Proved that DNA is the hereditary material |
| _____ l. Lederberg and Tatum | 12. Proved that microorganisms can cause disease |
| _____ m. Lister | 13. Said living cells arise from preexisting living cells |
| _____ n. Pasteur | 14. Showed that genes code for enzymes |
| _____ o. Stanley | 15. Spliced animal DNA to bacterial DNA |
| _____ p. van Leeuwenhoek | 16. Used bacteria to produce acetone |
| _____ q. Virchow | 17. Used the first synthetic chemotherapeutic agent |
| _____ r. Weizmann | 18. Proposed a classification system for streptococci based on antigens in their cell walls |

- The genus name of a bacterium is “erwinia,” and the specific epithet is “amylovora.” Write the scientific name of this organism correctly. Using this name as an example, explain how scientific names are chosen.
- It is possible to purchase the following microorganisms in a retail store. Provide a reason for buying each.
 - Bacillus thuringiensis*
 - Saccharomyces*
- DRAW IT** Show where airborne microbes ended up in Pasteur’s experiment.



- NAME IT** What type of microorganism has a peptidoglycan cell wall, has DNA that is not contained in a nucleus, and has flagella?

Multiple Choice

- Which of the following is a scientific name?
 - Mycobacterium tuberculosis*
 - Tubercle bacillus
- Which of the following is *not* a characteristic of bacteria?
 - are prokaryotic
 - have peptidoglycan cell walls
 - have the same shape
 - grow by binary fission
 - have the ability to move
- Which of the following is the most important element of Koch's germ theory of disease? The animal shows disease symptoms when
 - the animal has been in contact with a sick animal.
 - the animal has a lowered resistance.
 - a microorganism is observed in the animal.
 - a microorganism is inoculated into the animal.
 - microorganisms can be cultured from the animal.
- Recombinant DNA is
 - DNA in bacteria.
 - the study of how genes work.
 - the DNA resulting when genes of two different organisms are mixed.
 - the use of bacteria in the production of foods.
 - the production of proteins by genes.
- Which of the following statements is the best definition of *biogenesis*?
 - Nonliving matter gives rise to living organisms.
 - Living cells can only arise from preexisting cells.
 - A vital force is necessary for life.
 - Air is necessary for living organisms.
 - Microorganisms can be generated from nonliving matter.
- Which of the following is a beneficial activity of microorganisms?
 - Some microorganisms are used as food for humans.
 - Some microorganisms use carbon dioxide.
 - Some microorganisms provide nitrogen for plant growth.
 - Some microorganisms are used in sewage treatment processes.
 - all of the above
- It has been said that bacteria are essential for the existence of life on Earth. Which of the following is the essential function performed by bacteria?
 - control insect populations
 - directly provide food for humans
 - decompose organic material and recycle elements
 - cause disease
 - produce human hormones such as insulin
- Which of the following is an example of bioremediation?
 - application of oil-degrading bacteria to an oil spill
 - application of bacteria to a crop to prevent frost damage
 - fixation of gaseous nitrogen into usable nitrogen
 - production by bacteria of a human protein such as interferon
 - all of the above
- Spallanzani's conclusion about spontaneous generation was challenged because Lavoisier had just shown that oxygen was the vital component of air. Which of the following statements is true?
 - All life requires air.
 - Only disease-causing organisms require air.
 - Some microbes do not require air.
 - Pasteur kept air out of his biogenesis experiments.
 - Lavoisier was mistaken.
- Which of the following statements about *E. coli* is *false*?
 - E. coli* was the first disease-causing bacterium identified by Koch.
 - E. coli* is part of the normal microbiota of humans.
 - E. coli* is beneficial in human intestines.
 - A disease-causing strain of *E. coli* causes bloody diarrhea.
 - none of the above

Critical Thinking

- How did the theory of biogenesis lead the way for the germ theory of disease?
- Even though the germ theory of disease was not demonstrated until 1876, why did Semmelweis (1840) and Lister (1867) argue for the use of aseptic techniques?
- Find at least three supermarket products made by microorganisms. (*Hint: The label will state the scientific name of the organism or include the word culture, fermented, or brewed.*)
- People once believed all microbial diseases would be controlled by the twenty-first century. Name one emerging infectious disease. List three reasons why we are identifying new diseases now.

Clinical Applications

- The prevalence of arthritis in the United States is 1 in 100,000 children. However, 1 in 10 children in Lyme, Connecticut, developed arthritis between June and September 1973. Allen Steere, a rheumatologist at Yale University, investigated the cases in Lyme and found that 25% of the patients remembered having a skin rash during their arthritic episode and that the disease was treatable with penicillin. Steere concluded that this was a new infectious disease and did not have an environmental, genetic, or immunologic cause.
 - What was the factor that caused Steere to reach his conclusion?
 - What is the disease?
 - Why was the disease more prevalent between June and September?
- In 1864, Lister observed that patients recovered completely from simple fractures, but that compound fractures had "disastrous consequences." He knew that the application of phenol (carbolic acid) to fields in the town of Carlisle prevented cattle disease. Lister treated compound fractures with phenol, and his patients recovered without complications. How was Lister influenced by Pasteur's work? Why was Koch's work still needed?



2

Chemical Principles

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We can see a tree rot and smell milk going sour, but we might not realize what is happening on a microscopic level. In both cases, microbes are conducting chemical operations. The tree rots when microorganisms decompose the wood. Milk turns sour from the production of lactic acid by bacteria. Most of the activities of microorganisms are the result of a series of chemical reactions.

Like all organisms, microorganisms use nutrients to make chemical building blocks for growth and other functions essential to life. For most microorganisms, synthesizing these building blocks requires them to break down nutrient substances and use the energy released to assemble the resulting molecular fragments into new substances.

The chemistry of microbes is one of the most important concerns of microbiologists. Knowledge of chemistry is essential to understanding what roles microorganisms play in nature, how they cause disease, how methods for diagnosing disease are developed, how the body's defenses combat infection, and how antibiotics and vaccines are produced to combat the harmful effects of microbes. The *Bacillus anthracis* bacteria in the photograph make a capsule that is not readily digested by animal cells. As discussed in the Clinical Case, these bacteria can grow in mammals by avoiding host defenses. Researchers are investigating ways to identify unique chemicals made by *B. anthracis* and other potential biological weapons in order to detect bioterrorism. To understand the changes that occur in microorganisms and the changes microbes make in the world around us, we need to know how molecules are formed and how they interact.

The Structure of Atoms

LEARNING OBJECTIVE

2-1 Describe the structure of an atom and its relation to the physical properties of elements.

All matter—whether air, rock, or a living organism—is made up of small units called atoms. An **atom** is the smallest component of a pure substance that exhibits physical and chemical properties of that substance; an atom cannot be subdivided into smaller substances without losing its properties. Atoms interact with each other in certain combinations to form **molecules**. Living cells are made up of molecules, some of which are very complex. The science of the interaction between atoms and molecules is called **chemistry**.

Atoms are the smallest units of matter that enter into chemical reactions. Every atom has a centrally located **nucleus** and particles called **electrons** that move around the nucleus in regions called electron shells (**Figure 2.1**). The nuclei of most atoms are stable—that is, they do not change spontaneously—and nuclei do not participate in chemical reactions. The nucleus is made up of positively (+) charged particles called **protons** and uncharged (neutral) particles called **neutrons**. The nucleus, therefore, bears a net positive charge. A **charge** is a property of some subatomic particles that produces an attractive or repulsive force between them; particles of opposite charge attract each other, and particles of the same charge

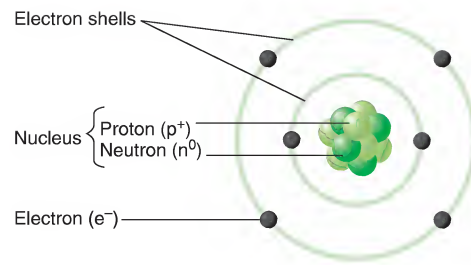


Figure 2.1 The structure of an atom. In this simplified diagram of a carbon atom, note the central location of the nucleus. The nucleus contains six neutrons and six protons, although not all the protons are visible in this view. The six electrons move about the nucleus in regions called electron shells, shown here as circles.

Q What is the atomic number of this atom?

repel each other. Neutrons and protons have approximately the same weight, which is about 1840 times that of an electron. The charge on electrons is negative (–), and in all atoms the number of electrons is equal to the number of protons. Because the total positive charge of the nucleus equals the total negative charge of the electrons, each atom is electrically neutral.

The number of protons in an atomic nucleus ranges from one (in a hydrogen atom) to more than 100 (in the largest atoms known). Atoms are often listed by their **atomic number**, the number of protons in the nucleus. The total number of protons and neutrons in an atom is its approximate **atomic weight**.

Chemical Elements

All atoms with the same number of protons behave the same way chemically and are classified as the same **chemical element**. Each element has its own name and a one- or two-letter symbol, usually derived from the English or Latin name for the element. For example, the symbol for the element hydrogen is H, and the symbol for carbon is C. The symbol for sodium is Na—the first two letters of its Latin name, *natrium*—to distinguish it from nitrogen, N, and from sulfur, S. There are 92 naturally occurring elements. However, only about 26 elements are commonly found in living things. **Table 2.1** lists some of the chemical elements found in living organisms.

Most elements have several **isotopes**—atoms with different numbers of neutrons in their nuclei. All isotopes of an element have the same number of protons in their nuclei, but their atomic weights differ because of the difference in the number of neutrons. For example, in a natural sample of oxygen, all the atoms contain eight protons. However, 99.76% of the atoms have eight neutrons, 0.04% contain nine neutrons, and the remaining 0.2% contain ten neutrons. Therefore, the three isotopes composing a natural sample of oxygen have atomic weights of 16, 17, and 18, although all will have the atomic number 8. Atomic numbers are written as a subscript to the left of an element's chemical

Clinical Case: Drumming Up Dust

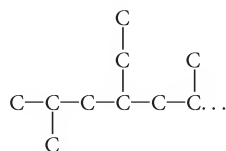
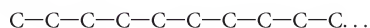
Jonathan, a 52-year-old drummer, is doing his best to ignore the cold sweat that is breaking out all over his body. He and his bandmates are performing in a local Philadelphia nightclub, and they are just about finished with the second set of the evening. Jonathan hasn't been feeling well for a while, actually; he has been feeling weak and short of breath for the last 3 days or so. Jonathan makes it to the end of the song, but the noise from the clapping and cheering audience seems to come from far away. He stands up to bow and collapses. Jonathan is admitted to a local emergency department with a mild fever and severe shaking. He is able to tell the admitting nurse that he also has had a dry cough for the last few days. The attending physician orders a chest X-ray exam and sputum culture. Jonathan is diagnosed with bilateral pneumonia caused by *Bacillus anthracis*. The attending physician is astonished by this diagnosis.

**How did Jonathan become infected by *B. anthracis*?
Read on to find out.**

26 43 44 48

Critical Thinking

- Here are the formulas of two detergents that have been manufactured:



Which of these would be resistant, and which would be readily degraded by microorganisms? (*Hint*: Refer to the degradation of fatty acids in Chapter 5.)

- Explain the effect of dumping untreated sewage into a pond on the eutrophication of the pond. The effect of sewage that has primary treatment? The effect of sewage that has secondary treatment? Contrast your previous answers with the effect of each type of sewage on a fast-moving river.

Clinical Applications

- Flooding after two weeks of heavy rainfall in Tooele, Utah, preceded a high rate of diarrheal illness. *G. lamblia* was isolated from 25% of the patients. A comparison study of a town 65 miles away revealed that there was diarrheal illness in 2.9% of the 103 people interviewed. Tooele has a municipal water system and a municipal sewage treatment plant. Explain the probable cause of this epidemic and method(s) of stopping it. What would a fecal coliform test have shown?
- The bioremediation process shown in the photograph is used to remove benzene and other hydrocarbons from soil contaminated by petroleum. The pipes are used to add nitrates, phosphates, oxygen, or water. Why are each of these added? Why is it not always necessary to add bacteria?



28

Applied and Industrial Microbiology

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In Chapter 27, we saw that microbes are an essential factor in many natural phenomena that make life possible on Earth. In this chapter we will look at how microorganisms are harnessed in such useful applications as the making of food and industrial products. Many of these processes—especially baking, winemaking, brewing, and cheesemaking—have origins long lost in history.

Modern civilization, with its large urban populations, could not be supported without methods of preserving food. In fact, civilization arose only after agriculture produced a year-round, stable food supply so that people were able to give up a nomadic hunting-and-gathering way of life.

In Chapter 9, we discussed industrial applications of genetically modified microorganisms that are at the cutting edge of our knowledge of molecular biology. Many of these applications are now essential to modern industry. (See the box in Chapter 1, page 3.) In this chapter we will explore the microbial production of foods, drugs, and chemicals. The Clinical Case shows the role of microbiologists in ensuring that pathogens such as *Salmonella* (in the photo) are not in foods.

Food Microbiology

LEARNING OBJECTIVES

- 28-1** Describe thermophilic anaerobic spoilage and flat sour spoilage by mesophilic bacteria.
- 28-2** Compare and contrast food preservation by industrial food canning, aseptic packaging, radiation, and high pressure.
- 28-3** Name four beneficial activities of microorganisms.

Many of the methods of food preservation used today were probably discovered by chance in centuries past. People in early cultures observed that dried meat and salted fish resisted decay. Nomads must have noticed that soured animal milk resisted further decomposition and was still palatable. Moreover, if the curd of the soured milk was pressed to remove moisture and allowed to ripen (in effect, cheesemaking), it was even more effectively preserved and tasted better. Farmers soon learned that if grains were kept dry, they did not become moldy.

Foods and Disease

As more food products are being prepared at central facilities and widely distributed, it is becoming more likely that food, like municipal water supplies, might be a source of widespread disease outbreaks. To minimize the potential for disease outbreaks, communities have established local agencies whose role is to inspect dairies and restaurants. The United States Food and Drug Administration (FDA) and Department of Agriculture (USDA) also maintain a system of inspectors at ports and central processing locations. A recent development in this field has been the introduction of the **Hazard Analysis and Critical Control Point (HACCP)** system, which is intended to safeguard food “from farm to fork.” Before the introduction of the HACCP system, the primary role of governmental agencies was to conduct sampling to identify contaminated foods. Such sampling to identify contamination will still have its place, but the HACCP system is designed to prevent contamination by identifying points at which foods are most likely to be contaminated with harmful microbes. Monitoring of these control points can prevent such microbes from being introduced or, if they are

present, arrest their proliferation. For example, the HACCP system can identify steps during processing at which meats are likely to become contaminated by the animal’s intestinal contents. The HACCP system also requires monitoring of adequate temperatures to kill pathogens during processing and adequate storage temperatures to prevent their reproduction.

Industrial Food Canning

In Chapter 7, you learned that preserving foods by heating a properly sealed container, as in home canning, is not difficult. The challenge in commercial canning is to use the right amount of heat necessary to kill spoilage organisms and dangerous microbes, such as the endospore-forming *Clostridium botulinum*, without degrading the appearance and palatability of food. Thus, much research is applied to determining the exact minimum heat treatment that will accomplish both these goals.

Industrial food canning is much more technically sophisticated than home canning (Figure 28.1). Industrially canned goods undergo **commercial sterilization** by steam under pressure in a large **retort** (Figure 28.2), which operates on the same principle as an autoclave (see Figure 7.2, page 186). Commercial sterilization is intended to destroy *C. botulinum* endospores and is not as rigorous as complete sterilization. The reasoning is that if *C. botulinum* endospores are destroyed, then any other significant spoilage or pathogenic bacteria will also be destroyed.

To ensure commercial sterilization, enough heat is applied for the **12D treatment** (12-decimal reductions, or *botulininal cook*), by which a theoretical population of *C. botulinum* endospores would be decreased by 12 logarithmic cycles. (See Figure 7.1 and Table 7.2, page 183.) What this means is that if there were 10^{12} (1,000,000,000,000) endospores in a can, after treatment there would be only one survivor. Because 10^{12} is an improbably large population, this treatment is considered quite safe. Certain thermophilic endospore-forming bacteria have endospores that are more resistant to heat treatment than those of *C. botulinum*. However, these bacteria are obligate thermophiles and generally remain dormant at temperatures lower than about 45°C. Therefore, they are not a spoilage problem at normal storage temperatures.

Spoilage of Canned Food

If canned foods are incubated at high temperatures, such as in a truck in the hot sun or next to a steam radiator, the thermophilic bacteria that often survive commercial sterilization can germinate and grow. **Thermophilic anaerobic spoilage** is therefore a fairly common cause of spoilage in low-acid canned foods. The can usually swells from gas, and the contents have a lowered pH and a sour odor. A number of thermophilic species of *Clostridium* can cause this type of spoilage. When thermophilic spoilage occurs but the can is not swollen by gas production, the spoilage is termed **flat sour spoilage**. This type

Clinical Case: Dr. Chang and the Chocolate Factory

Dr. Derrick Chang of the CDC is alerted by PulseNet, the national molecular subtyping network for foodborne disease surveillance. PulseNet has identified an increase of genetically identical *Salmonella typhimurium* in the United States. This increase shows 120 isolates from 23 states in the last 60 days.

What is causing this outbreak? Read on to find out.

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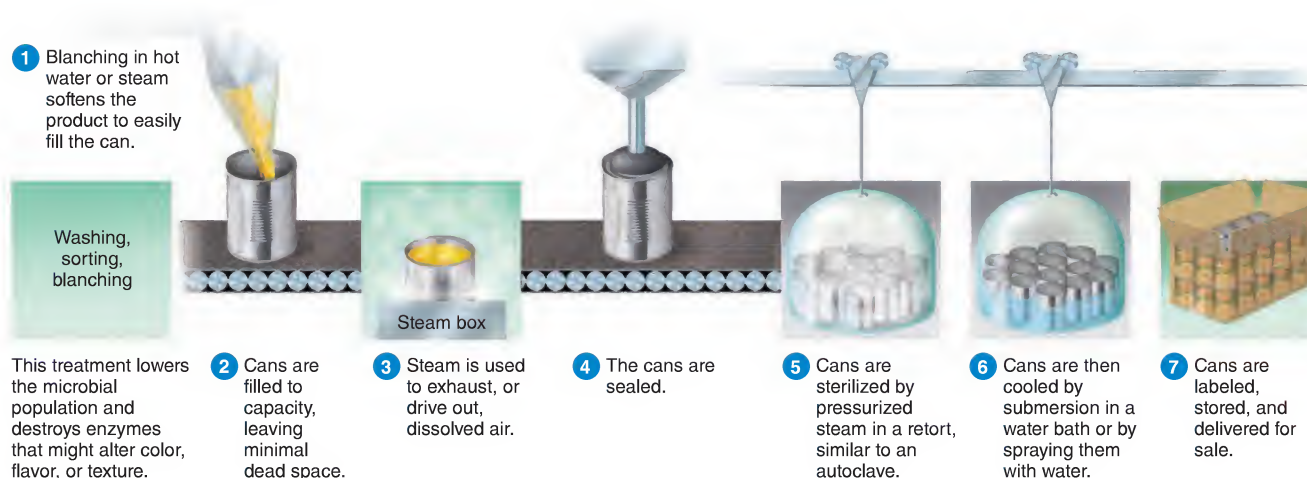


Figure 28.1 The commercial sterilization process in industrial canning.

Q How does commercial sterilization differ from complete sterilization?

of spoilage is caused by thermophilic organisms such as *Geobacillus stearothermophilus* (ste-rō-thēr-mă'fil-us), which is found in the starch and sugars used in food preparation. Many industries have standards for the numbers of such thermophilic bacteria permitted in raw materials. Both types of spoilage occur only when the cans are stored at higher than normal temperatures, which permits the growth of bacteria whose endospores are not destroyed by normal processing.

Mesophilic bacteria can spoil canned foods if the food is underprocessed or if the can leaks. Underprocessing is more likely to result in spoilage by endospore formers; the presence of non-endospore-forming bacteria strongly suggests that the can leaks. Leaking cans are often contaminated during the cooling of cans after processing by heat. The hot cans are sprayed with cooling water or passed through a trough filled with water. As the can cools, a vacuum is formed inside, and external water can be sucked through a leak past the heat-softened sealant in the crimped lid (Figure 28.3). Contaminating bacteria in the cooling water are drawn into the can with the water. Spoilage from underprocessing or can leakage is likely to produce odors of putrefaction, at least in high-protein foods, and occurs at normal storage temperatures. In such types of spoilage, there is always the potential that botulinal bacteria will be present.

Some acidic foods, such as tomatoes or preserved fruits, are preserved by processing temperatures of 100°C or lower. The reasoning is that the only spoilage organisms that will grow in such acidic foods are easily killed by even 100°C temperatures. Primarily, these would be molds, yeasts, and certain vegetative bacteria.

Occasional problems in acidic foods develop from a few microorganisms that are both heat-resistant and acid-tolerant.

Examples of heat-resistant fungi are the mold *Byssoschlamys fulva* (bis-sō-klam'is ful'vā), which produces a *heat-resistant ascospore*, and a few molds, especially species of *Aspergillus*, that sometimes produce specialized resistant bodies called *sclerotia*. A spore-forming bacterium, *Bacillus coagulans* (kō-ag'ū-lanz), is unusual in that it is capable of growth at a pH of almost 4.0. Table 28.1 lists types of spoilage in low- and medium-acid foods.

Aseptic Packaging

The use of **aseptic packaging** to preserve food has been increasing. Packages are usually made of some material that cannot



Figure 28.2 Commercial canning retorts. These are much larger than the sterilizing autoclaves used in most microbiology laboratories or hospitals.

Q Is there any difference in principle between a canning retort and a hospital autoclave?

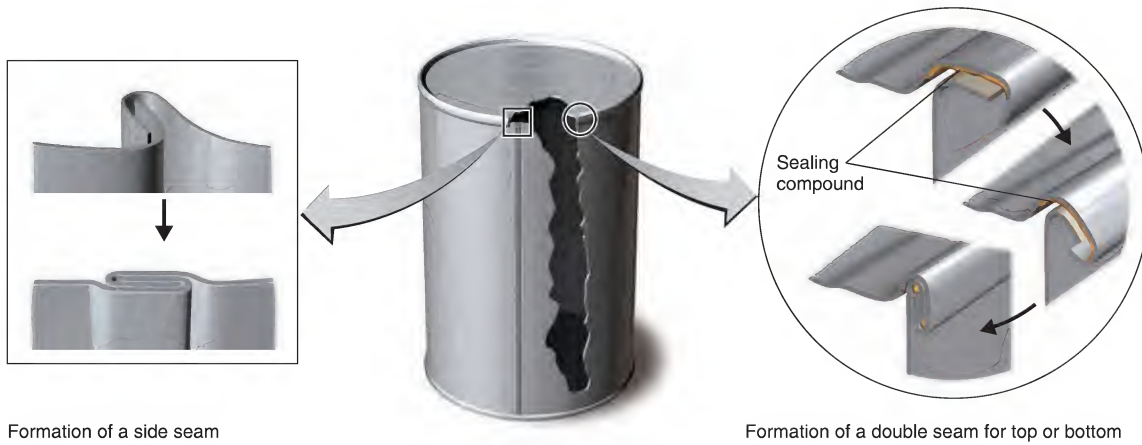


Figure 28.3 The construction of a metal can. Notice the seam construction, which was introduced about 1904. During cooling after sterilization (see Figure 28.1, step 6), the vacuum formed in the can may actually force contaminating organisms into the can along with water.

Q Why isn't the can sealed before it is placed in the steam box?

tolerate conventional heat treatment, such as laminated paper or plastic. The packaging materials come in continuous rolls that are fed into a machine that sterilizes the material with a hot hydrogen peroxide solution, sometimes aided by ultraviolet (UV) light (Figure 28.4). Metal containers can be sterilized with superheated steam or other high-temperature methods. High-energy electron

beams can also be used to sterilize the packaging materials. While still in the sterile environment, the material is formed into packages, which are then filled with liquid foods that have been conventionally sterilized by heat. The filled package is not sterilized after it is sealed.



Figure 28.4 Aseptic packaging. Rolls of packaging material in foreground, filled packages at right center.

Q Why has the use of this procedure been increasing in recent years?

Clinical Case

Dr. Chang initiates a case-control study with representatives of the state health departments that had reported *S. typhimurium* infections. Fifteen items, suspected as possible vehicles of infection on the basis of the individual case investigations, are listed. State officials determine whether each suspect item was used or consumed by the infected person within the 3 days before onset of illness. The family of each patient identifies two neighborhood controls, of the same age and gender as the patient. Controls were asked the same questions as patients, except that they were questioned about the use or consumption of the 15 suspect items during the previous month. Some of the data collected are shown in the table.

Foil-Wrapped Chocolate Balls	Cases	Controls
Ate	38	12
Did not eat	7	79

Calculate the relative risk for this food item.
(Hint: See page 721)

800 802 807 811 813 815

TABLE 28.1 Common Types of Spoilage in Low-Acid and Medium-Acid Canned Foods (pH above 4.5)

Type of Spoilage	Indications of Spoilage	
	Appearance of Can	Contents of Can
Flat sour (<i>Geobacillus stearothermophilus</i>)	Can not swollen	Appearance not usually altered; pH markedly lowered; sour; may have slightly abnormal odor; sometimes cloudy liquid
Thermophilic anaerobic (<i>Thermoanaerobacterium thermosaccharolyticum</i>)	Swollen	Fermented, sour, cheesy, or butyric acid odor
Putrefactive anaerobic (<i>Clostridium sporogenes</i> ; possibly <i>C. botulinum</i>)	Swollen	May be partially digested; pH slightly above normal; typical putrid odor

Radiation and Industrial Food Preservation

It has long been recognized that irradiation is lethal to microorganisms; in fact, a patent was issued in Great Britain in 1905 for the use of ionizing radiation to improve the condition of foodstuffs. X rays were specifically suggested in 1921 as a way to inactivate the larvae in pork that are the cause of trichinellosis. Ionizing irradiation inhibits DNA synthesis and effectively prevents microorganisms, insects, and plants from reproducing. The ionizing irradiation is usually X rays or the gamma rays produced by radioactive cobalt-60. Up to certain energy levels, high energy electrons produced by electron accelerators are also used. The main practical difference is in penetration capabilities. These sources inactivate the target organisms and do *not* induce radioactivity in the food or packaging material. The relative doses of radiation needed to kill various organisms are presented in [Table 28.2](#). Radiation is measured in *Grays*, named for an early radiologist—often in terms of thousands of Grays, abbreviated as kGy.

- *Low doses of irradiation (less than 1 kGy)* are used for killing insects (disinfestation) and inhibiting sprouting, as in stored potatoes. Similarly, it can delay ripening of fruits during storage.

- *Pasteurizing doses (1 to 10 kGy)* can be used on meats and poultry to eliminate or critically reduce the numbers of specific bacterial pathogens.
- *High doses (more than 10 kGy)* are used to sterilize, or at least greatly lower, the bacterial populations in many spices. Spices are often contaminated with 1 million or more bacteria per gram, although these are not considered to be normally hazardous to health.

A specialized use of irradiation has been to sterilize meats eaten by American astronauts, and a few health facilities have selectively used irradiation to sterilize foods ingested by immunocompromised patients. Millions of implanted medical devices, such as pacemakers, have been irradiated. Irradiated food is marked in the United States with a radura symbol ([Figure 28.5](#)) and a printed notice. Unfortunately, this symbol has often been interpreted as a warning rather than the description of an approved processing treatment or preservative. In fact, irradiated foods are not radioactive; consider that the X-ray table in a hospital does not become radioactive from repeated daily exposure to ionizing radiation. Recently, the FDA has allowed, upon special approval, substitution of language such as “pasteurization” rather than “irradiation.”

When deep penetration is a requirement, the preferred method for irradiation is gamma rays produced by cobalt-60.

TABLE 28.2 Approximate Doses of Radiation Needed to Kill Various Organisms (Prions Are Not Affected)

Organisms	Dose (kGy)*
Higher animals (whole body)	0.005–0.1
Insects	0.01–1
Non-endospore-forming bacteria	0.5–10
Bacterial endospores	10–50
Viruses	10–200

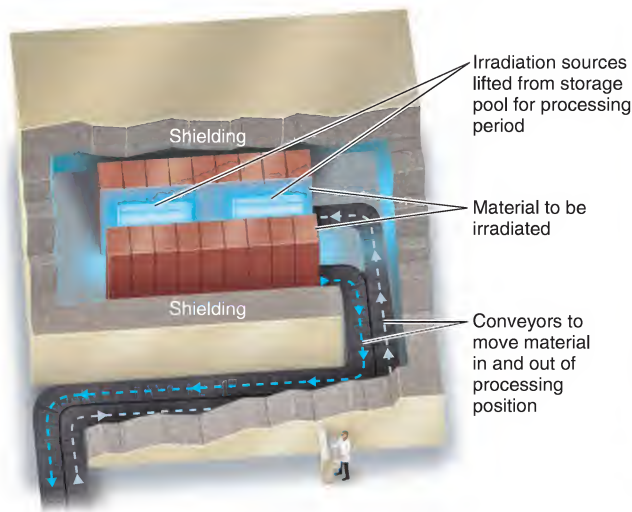
*Gray is a measure of ionizing irradiation; kGy is 1000 Grays.

Source: J. Farkas, “Physical Methods of Food Preservation,” in *Food Microbiology: Fundamentals and Frontiers*, 2d ed., M.P. Doyle et al. (eds) (Washington, DC: ASM Press, 2001).

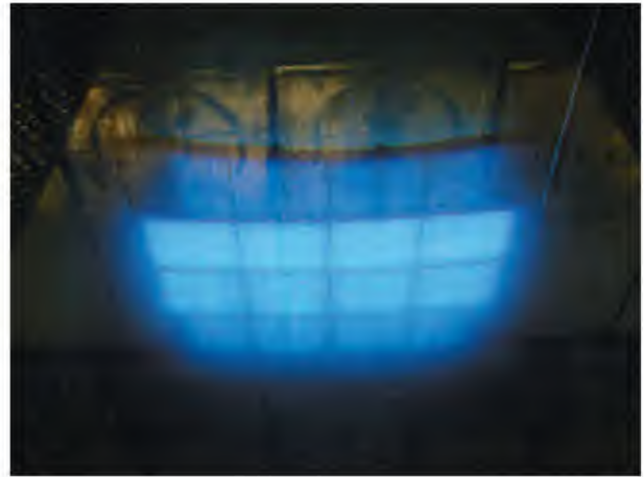


Figure 28.5 Irradiation logo. This logo, the international radura symbol, indicates that a food has received irradiation treatment.

Q Is irradiation the same as a chemical additive?



(a) An irradiation facility, showing the path of the material to be irradiated



(b) The irradiation source is submerged in the storage pool. The blue glow is Cerenkov radiation caused by charged particles exceeding the speed of light in water.

Figure 28.6 A gamma-ray irradiation facility.

Q Can microwaves be used to sterilize foods?

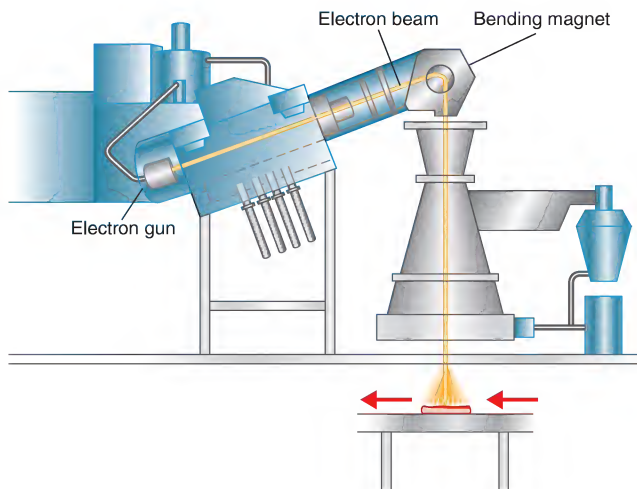


Figure 28.7 Electron-beam accelerator. These machines generate an electron stream that is accelerated down a long tube by electromagnets of the opposite charge. In the drawing, the electron beam is bent by a "bending magnet." This serves to filter out electrons of unwanted energy levels, providing a beam of uniform energy. The vertical beam is swept back and forth over the target as it is moved past the beam. The penetrating power of the beam is limited: if the target substance is expressed as an equivalent thickness of water, the maximum is about 3.9 cm (1.5 in). In contrast, X rays will penetrate about 23 cm (9 in).

Q Are high-energy electrons ionizing radiation?

However, this type of treatment requires several hours of exposure in isolation behind protective walls (**Figure 28.6**).

High-energy electron accelerators (**Figure 28.7**) are much faster and sterilize in a few seconds, but this treatment has low penetrating power and is suitable only for sliced meats, bacon, or similar thin products. Also, plasticware used in microbiology is usually sterilized in this way. Another recent application is to irradiate mail to kill possible bioterrorism agents that it might contain, such as anthrax endospores.

High-Pressure Food Preservation

A recent development in food preservation (pascalation) has been the use of a high-pressure processing technique. Prewrapped foods such as fruits, deli meats, and precooked chicken strips are submerged into tanks of pressurized water. The pressure can reach 87,000 pounds per square inch (psi)—which has been compared to the equivalent of about three elephants standing on a dime. This process kills many bacteria, such as *Salmonella*, *Listeria*, and pathogenic strains of *E. coli*, by disrupting many cellular functions. It also kills nonpathogenic microorganisms that tend to shorten the shelf life of such products.

Because the process does not require additives, it does not require regulatory approval. It has the advantage of preserving colors and tastes of foods better than many other methods and does not provoke the concerns of irradiation.

GLOSSARY

9 + 2 array Attachment of microtubules in eukaryotic flagella and cilia; 9 pairs of microtubules plus two microtubules.

12D treatment A sterilization process that would result in a decrease of the number of *Clostridium botulinum* endospores by 12 logarithmic cycles.

ABO blood group system The classification of red blood cells based on the presence or absence of A and B carbohydrate antigens.

abscess A localized accumulation of pus.

A-B toxin Bacterial exotoxins consisting of two polypeptides.

acellular vaccine A vaccine consisting of antigenic parts of cells.

acetyl group

$$\begin{array}{c} \text{O} \\ || \\ \text{H}_3\text{C}-\text{C}- \end{array}$$

acid A substance that dissociates into one or more hydrogen ions (H^+) and one or more negative ions.

acid-fast stain A differential stain used to identify bacteria that are not decolorized by acid-alcohol.

acidic dye A salt in which the color is in the negative ion; used for negative staining.

acidophile A bacterium that grows below pH 4.

acquired immunodeficiency The inability, obtained during the life of an individual, to produce specific antibodies or T cells, due to drugs or disease.

activated macrophage A macrophage that has increased phagocytic ability and other functions after exposure to mediators released by T cells after stimulation by antigens.

activated sludge system A process used in secondary sewage treatment in which batches of sewage are held in highly aerated tanks; to ensure the presence of microbes efficient in degrading sewage, each batch is inoculated with portions of sludge from a previous batch.

activation energy The minimum collision energy required for a chemical reaction to occur.

active site A region on an enzyme that interacts with the substrate.

active transport Net movement of a substance across a membrane against a concentration gradient; requires the cell to expend energy.

acute disease A disease in which symptoms develop rapidly but last for only a short time.

acute-phase proteins Serum proteins whose concentration changes by at least 25% during inflammation.

adaptive immunity The ability, obtained during the life of the individual, to produce specific antibodies and T cells.

adenosarcoma Cancer of glandular epithelial tissue.

adenosine diphosphate (ADP) The substance formed when ATP is hydrolyzed and energy is released.

adenosine triphosphate (ATP) An important intracellular energy source.

adherence Attachment of a microbe or phagocyte to another's plasma membrane or other surface.

adhesin A carbohydrate-specific binding protein that projects from prokaryotic cells; used for adherence, also called a ligand.

adjuvant A substance added to a vaccine to increase its effectiveness.

aerobe An organism requiring molecular oxygen (O_2) for growth.

aerobic respiration Respiration in which the final electron acceptor in the electron transport chain is molecular oxygen (O_2).

aerotolerant anaerobe An organism that does not use molecular oxygen (O_2) but is not affected by its presence.

aflatoxin A carcinogenic toxin produced by *Aspergillus flavus*.

agar A complex polysaccharide derived from a marine alga and used as a solidifying agent in culture media.

agglutination A joining together or clumping of cells.

agranulocyte A leukocyte without visible granules in the cytoplasm; includes monocytes and lymphocytes.

alarmone A chemical signal that promotes a cell's response to environmental stress.

alcohol An organic molecule with the functional group—OH.

alcohol fermentation A catabolic process, beginning with glycolysis, that produces ethyl alcohol to reoxidize NADH.

aldehyde An organic molecule with the functional group



alga (plural: **algae**) A photosynthetic eukaryote; may be unicellular, filamentous, or multicellular but lack the tissues found in plants.

algal bloom An abundant growth of microscopic algae producing visible colonies in nature.

algin A sodium salt of mannuronic acid ($\text{C}_6\text{H}_8\text{O}_6$); found in brown algae.

allergen An antigen that evokes a hypersensitivity response.

allergy See hypersensitivity.

allograft A tissue graft that is not from a genetically identical donor (i.e., not from self or an identical twin).

allosteric inhibition The process in which an enzyme's activity is changed because of binding to the allosteric site.

allosteric site The site on an enzyme at which a noncompetitive inhibitor binds.

allylamines Antifungal agents that interfere with sterol synthesis.

amanitin A polypeptide toxin produced by *Amanita* spp., inhibits RNA polymerase.

Ames test A procedure using bacteria to identify potential carcinogens.

amination The addition of an amino group.

amino acid An organic acid containing an amino group and a carboxyl group. In alpha-amino acids the amino and carboxyl groups are attached to the same carbon atom called the alpha-carbon.

aminoglycoside An antibiotic consisting of amino sugars and an aminocyclitol ring; for example, streptomycin.

amino group $-\text{NH}_2$.

ammonification The release of ammonia from nitrogen-containing organic matter by the action of microorganisms.

amphibolic pathway A pathway that is both anabolic and catabolic.

amphitrichous Having flagella at both ends of a cell.

anabolism All synthesis reactions in a living organism; the building of complex organic molecules from simpler ones.

anaerobe An organism that does not require molecular oxygen (O_2) for growth.

anaerobic respiration Respiration in which the final electron acceptor in the electron transport chain is an inorganic molecule other than molecular oxygen (O_2); for example, a nitrate ion or CO_2 .

anaerobic sludge digester Anaerobic digestion used in secondary sewage treatment.

anal pore A site in certain protozoa for elimination of waste.

analytical epidemiology Comparison of a diseased group and a healthy group to determine the cause of the disease.

anamnesic response See memory response.

anamorph Ascomycete fungi that have lost the ability to reproduce sexually; the asexual stage of a fungus.

anaphylaxis A hypersensitivity reaction involving IgE antibodies, mast cells, and basophils.

Angstrom (\AA) A unit of measurement equal to 10^{-10} m, or 0.1 nm.

Animalia The kingdom composed of multicellular eukaryotes lacking cell walls.

anion An ion with a negative charge.

anoxygenic Not producing molecular oxygen; typical of cyclic photophosphorylation.

antagonism Active opposition; (1) When two drugs are less effective than either one alone. (2) Competition among microbes.

antibiogram Report of antibiotic susceptibility of a bacterium.

antibiotic An antimicrobial agent, usually produced naturally by a bacterium or fungus.

antibody A protein produced by the body in response to an antigen, and capable of combining specifically with that antigen.

antibody-dependent cell-mediated cytotoxicity (ADCC) The killing of antibody-coated cells by natural killer cells and leukocytes.

antibody titer The amount of antibody in serum.

anticodon The three nucleotides by which a tRNA recognizes an mRNA codon.

antigen Any substance that causes antibody formation; also called immunogen.

antigen-antibody complex The combination of an antigen with the antibody that is specific for it; the basis of immune protection and many diagnostic tests.

antigen-binding sites A site on an antibody that binds to an antigenic determinant.

antigenic determinant A specific region on the surface of an antigen against which antibodies are formed; also called epitope.

antigenic drift A minor variation in the antigenic makeup of influenza viruses that occurs with time.

antigenic shift A major genetic change in influenza viruses causing changes in H and N antigens.

antigenic variation Changes in surface antigens that occur in a microbial population.

antigen-presenting cell (APC) A macrophage, dendritic cell, or B cell that engulfs an antigen and presents fragments to T cells.

anti-human immune serum globulin (anti-HISG) An antibody that reacts specifically with human antibodies.

antimetabolite A competitive inhibitor.

antimicrobial peptide An antibiotic that is bactericidal and has a broad spectrum of activity; see bacteriocin.

antisense DNA DNA that is complementary to the DNA encoding a protein; the antisense RNA transcript will hybridize with the mRNA encoding the protein and inhibit synthesis of the protein.

antisense strand (– strand) Viral RNA that cannot act as mRNA.

antiseptic A chemical method for disinfection of the skin or mucous membranes; the chemical is called an antiseptic.

antiserum A blood-derived fluid containing antibodies.

antitoxin A specific antibody produced by the body in response to a bacterial exotoxin or its toxoid.

antiviral protein (AVP) A protein made in response to interferon that blocks viral multiplication.

apoenzyme The protein portion of an enzyme, which requires activation by a coenzyme.

apoptosis The natural programmed death of a cell; the residual fragments are disposed of by phagocytosis.

aquatic microbiology The study of microorganisms and their activities in natural waters.

arbuscule Fungal mycelia in plant root cells.

archaea Domain of prokaryotic cells lacking peptidoglycan; one of the three domains.

arthroconidia An asexual fungal spore formed by fragmentation of a septate hypha.

Arthus reaction Inflammation and necrosis at the site of injection of foreign serum, due to immune complex formation.

artificially acquired active immunity The production of antibodies by the body in response to a vaccination.

artificially acquired passive immunity The transfer of humoral antibodies formed by one individual to a susceptible individual, accomplished by the injection of antiserum.

artificial selection Choosing one organism from a population to grow because of its desirable traits.

ascospore A sexual fungal spore produced in an ascus, formed by the ascomycetes.

ascus A saclike structure containing ascospores; found in the ascomycetes.

asepsis The absence of contamination by unwanted organisms.

aseptic packaging Commercial food preservation by filling sterile containers with sterile food.

aseptic surgery Techniques used in surgery to prevent microbial contamination of the patient.

aseptic techniques Laboratory techniques used to minimize contamination.

asexual spore A reproductive cell produced by mitosis and cell division (eukaryotes) or binary fission (actinomycetes).

atom The smallest unit of matter that can enter into a chemical reaction.

atomic force microscopy See scanned-probe microscopy.

atomic number The number of protons in the nucleus of an atom.

atomic weight The total number of protons and neutrons in the nucleus of an atom.

atrichous Bacteria that lack flagella.

attenuated vaccine A vaccine containing live, attenuated (weakened) microorganisms.

autoclave Equipment for sterilization by steam under pressure, usually operated at 15 psi and 121°C.

autograft A tissue graft from one's self.

autoimmune disease Damage to one's own organs due to action of the immune system.

autotroph An organism that uses carbon dioxide (CO₂) as its principal carbon source. chemoautotroph, photoautotroph.

auxotroph A mutant microorganism with a nutritional requirement that is absent in the parent.

axial filament The structure for motility found in spirochetes; also called endoflagellum.

azole Antifungal agents that interfere with sterol synthesis.

bacillus (plural: **bacilli**) (1) Any rod-shaped bacterium. (2) When written as a genus (*Bacillus*) refers to rod-shaped, endospore-forming, facultatively anaerobic, gram-positive bacteria.

bacteremia A condition in which there are bacteria in the blood.

bacteria Domain of prokaryotic organisms, characterized by peptidoglycan cell walls; **bacterium** (singular) when referring to a single organism.

bacterial growth curve A graph indicating the growth of a bacterial population over time.

bactericide A substance capable of killing bacteria.

bacteriocin An antimicrobial peptide produced by bacteria that kills other bacteria.

bacteriochlorophyll A photosynthetic pigment that transfers electrons for photophosphorylation; found in anoxygenic photosynthetic bacteria.

bacteriology The scientific study of prokaryotes, including bacteria and archaea.

bacteriophage (phage) A virus that infects bacterial cells.

bacteriostasis A treatment capable of inhibiting bacterial growth.

base A substance that dissociates into one or more hydroxide ions (OH[–]) and one or more positive ions.

base pairs The arrangement of nitrogenous bases in nucleic acids based on hydrogen bonding; in DNA, base pairs are A-T and G-C; in RNA, base pairs are A-U and G-C.

base substitution The replacement of a single base in DNA by another base, causing a mutation; also called point mutation.

basic dye A salt in which the color is in the positive ion; used for bacterial stains.

basidiospore A sexual fungal spore produced in a basidium, characteristic of the basidiomycetes.

basidium A pedestal that produces basidiospores; found in the basidiomycetes.

basophil A granulocyte (leukocyte) that readily takes up basic dye and is not phagocytic; has receptors for IgE Fc regions.

batch production An industrial process in which cells are grown for a period of time after which the product is collected.

B cell A type of lymphocyte; differentiates into antibody-secreting plasma cells and memory cells.

BCG vaccine A live, attenuated strain of *Mycobacterium bovis* used to provide immunity to tuberculosis.

beer Alcoholic beverage produced by fermentation of starch.

benthic zone The sediment at the bottom of a body of water.

Bergey's Manual *Bergey's Manual of Systematic Bacteriology*, the standard taxonomic reference on bacteria; also refers to *Bergey's Manual of Determinative Bacteriology*, the standard laboratory identification reference on bacteria.

purple sulfur bacteria Gammaproteobacteria; strictly anaerobic and phototrophic; use reduced sulfur compounds as electron donors for CO₂ fixation.

pus An accumulation of dead phagocytes, dead bacterial cells, and fluid.

pustule A small pus-filled elevation of skin.

pyocyanin A blue-green pigment produced by *Pseudomonas aeruginosa*.

pyrimidines The class of nucleic acid bases that includes uracil, thymine, and cytosine.

quaternary ammonium compound (quat) A cationic detergent with four organic groups attached to a central nitrogen atom; used as a disinfectant.

quorum sensing The ability of bacteria to communicate and coordinate behavior via signaling molecules.

R Used to represent nonfunctional groups of a molecule. *See also* resistance factor.

rapid diagnostic test (RDT) A test that allows diagnosis of a disease within a few minutes.

rapid identification methods Bacterial identification tools that perform several biochemical tests simultaneously.

rapid plasma reagin (RPR) test A serological test for syphilis.

r-determinant A group of genes for antibiotic resistance carried on R factors.

RecA Catalyzes joining of DNA strands, facilitates recombination of DNA.

receptor An attachment for a pathogen on a host cell.

receptor-mediated endocytosis A type of pinocytosis in which molecules bound to proteins on the plasma membrane are taken in by infolding of the membrane.

recipient cell A cell that receives DNA from a donor cell during genetic recombination.

recombinant DNA (rDNA) A DNA molecule produced by combining DNA from two different sources.

recombinant DNA (rDNA) technology Manufacturing and manipulating genetic material in vitro; also called genetic engineering.

recombinant vaccine A vaccine made by recombinant DNA techniques.

redia A trematode larval stage that reproduces asexually to produce cercariae.

redox reaction *See* oxidation-reduction.

red tide A bloom of planktonic dinoflagellates.

reducing medium A culture medium containing ingredients that will remove dissolved oxygen from the medium to allow the growth of anaerobes.

reduction The addition of electrons to a molecule.

refractive index The relative velocity with which light passes through a substance.

relative risk A comparison of the risk of disease in two groups.

rennin An enzyme that forms curds as part of any dairy fermentation product; originally from calves' stomachs, now produced by molds and bacteria.

replica plating A method of inoculating a number of solid minimal culture media from an original plate to produce the same pattern of colonies on each plate.

replication fork The point where DNA strands separate and new strands will be synthesized.

repression The process by which a repressor protein can stop the synthesis of a protein.

repressor A protein that binds to the operator site to prevent transcription.

reservoir of infection A continual source of infection.

resistance The ability to ward off diseases through innate and adaptive immunity.

resistance (R) factor A bacterial plasmid carrying genes that determine resistance to antibiotics.

resistance transfer factor (RTF) A group of genes for replication and conjugation on the R factor.

resolution The ability to distinguish fine detail with a magnifying instrument; also called resolving power.

respiration A series of redox reactions in a membrane that generates ATP; the final electron acceptor is usually an inorganic molecule.

restriction enzyme An enzyme that cuts double-stranded DNA at specific sites between nucleotides.

reticulate body The intracellular growing stage of chlamydiae.

reticuloendothelial system *See* mononuclear phagocytic system.

retort A device for commercially sterilizing canned food by using steam under pressure; operates on the same principle as an autoclave but is much larger.

reverse genetics Genetic analysis that begins with a piece of DNA and proceeds to find out what it does.

reverse transcriptase An RNA-dependent DNA polymerase; an enzyme that synthesizes a complementary DNA from an RNA template.

reversible reaction A chemical reaction in which the end-products can readily revert to the original molecules.

RFLP Restriction fragment length polymorphism; a fragment resulting from restriction-enzyme digestion of DNA.

Rh factor An antigen on red blood cells of rhesus monkeys and most humans; possession makes the cells Rh⁺.

rhizine A rootlike hypha that anchors a fungus to a surface.

ribonucleic acid (RNA) The class of nucleic acids that comprises messenger RNA, ribosomal RNA, and transfer RNA.

ribose A five-carbon sugar that is part of ribonucleotide molecules and RNA.

ribosomal RNA (rRNA) The type of RNA molecule that forms ribosomes.

ribosomal RNA (rRNA) sequencing Determination of the order of nucleotide bases in rRNA.

ribosome The site of protein synthesis in a cell, composed of RNA and protein.

ribotyping Classification or identification of bacteria based on rRNA genes.

ribozyme An enzyme consisting of RNA that specifically acts on strands of RNA to remove introns and splice together the remaining exons.

ring stage A young *Plasmodium* trophozoite that looks like a ring in a red blood cell.

RNAi RNA interference; stops gene expression at transcription by using a short interfering RNA to make double-stranded RNA.

RNA-induced silencing complex (RISC) A complex consisting of a protein and siRNA or miRNA that binds complementary mRNA, preventing transcription of the mRNA.

RNA primer A short strand of RNA used to start synthesis of the lagging strand of DNA, and to start the polymerase chain reaction.

root nodule A tumorlike growth on the roots of certain plants containing symbiotic nitrogen-fixing bacteria.

rotating biological contactor A method of secondary sewage treatment in which large disks are rotated while partially submerged in a sewage tank exposing sewage to microorganisms and aerobic conditions.

rough ER Endoplasmic reticulum with ribosomes on its surface.

roundworm An animal belonging to the phylum Nematoda.

S (Svedberg unit) Notes the relative rate of sedimentation during ultra-high speed centrifugation.

salt A substance that dissolves in water to cations and anions, neither of which is H⁺ or OH⁻.

sanitization The removal of microbes from eating utensils and food preparation areas.

saprophyte An organism that obtains its nutrients from dead organic matter.

sarcina (plural: **sarcinae**) (1) A group of eight bacteria that remain in a packet after dividing. (2) When written as a genus, refers to gram-positive, anaerobic cocci.

saturation (1) The condition in which the active site on an enzyme is occupied by the substrate or product at all times. (2) In a fatty acid, having no double bonds.

saxitoxin A neurotoxin produced by some dinoflagellates.

scanned-probe microscopy Microscopic technique used to obtain images of molecular shapes, to characterize chemical properties, and to determine temperature variations within a specimen.

scanning acoustic microscope (SAM) A microscope that uses high-frequency ultrasound waves to penetrate surfaces.

scanning electron microscope (SEM) An electron microscope that provides three-dimensional views of the specimen magnified 1000–10,000×

scanning tunneling microscopy *See* scanned-probe microscopy.

schizogony The process of multiple fission, in which one organism divides to produce many daughter cells.

scientific nomenclature *See* binomial nomenclature.

sclerotia The compact mass of hardened mycelia of the fungus *Claviceps purpurea* that fills infected rye flowers; produces the toxin ergot.

scolex The head of a tapeworm, containing suckers and possibly hooks.

secondary infection An infection caused by an opportunistic microbe after a primary infection has weakened the host's defenses.

secondary metabolite A product of an industrial cell population produced after the microorganism has largely completed its period of rapid growth and is in a stationary phase of the growth cycle. *See also* primary metabolite.

secondary response *See* memory response.

secondary sewage treatment Biological degradation of the organic matter in wastewater following primary treatment.

secretory vesicle A membrane-enclosed sac produced by the ER; transports synthesized material into cytoplasm.

selective medium A culture medium designed to suppress the growth of unwanted microorganisms and encourage the growth of desired ones.

selective permeability The property of a plasma membrane to allow certain molecules and ions to move through the membrane while restricting others.

selective toxicity The property of some antimicrobial agents to be toxic for a microorganism and nontoxic for the host.

self Host tissue.

semiconservative replication The process of DNA replication in which each double-stranded DNA molecule contains one original strand and one new strand.

sense codon A codon that codes for an amino acid.

sense strand (+ strand) Viral RNA that can act as mRNA.

sensitivity Percentage of positive samples correctly detected by a diagnostic test.

sentinel animal An organism in which changes can be measured to assess the extent of environmental contamination and its implication for human health.

sepsis The presence of a toxin or pathogenic organism in blood and tissue.

septate hypha A hypha consisting of uninucleate cell-like units.

septicemia The proliferation of pathogens in the blood, accompanied by fever; sometimes causes organ damage.

septic shock A sudden drop in blood pressure induced by bacterial toxins.

septum A cross-wall in a fungal hypha.

serial dilution The process of diluting a sample several times.

seroconversion A change in a person's response to an antigen in a serological test.

serological testing Techniques for identifying a microorganism based on its reaction with antibodies.

serology The branch of immunology that studies blood serum and antigen-antibody reactions in vitro.

serotype *See* serovar.

serovar A variation within a species; also called serotype.

serum The liquid remaining after blood plasma is clotted; contains antibodies (immunoglobulins).

sexual dimorphism The distinctly different appearance of adult male and female organisms.

sexual spore A spore formed by sexual reproduction.

Shiga toxin An exotoxin produced by *Shigella dysenteriae* and enterohemorrhagic *E. coli*.

shock Any life-threatening loss of blood pressure. *See also* septic shock.

short tandem repeats (STRs) Repeating sequences of 2- to 5-nucleotides.

shotgun sequencing A technique for determining the nucleotide sequence in an organism's genome.

shuttle vector A plasmid that can exist in several different species; used in genetic engineering.

siderophore Bacterial iron-binding proteins.

sign A change due to a disease that a person can observe and measure.

simple stain A method of staining microorganisms with a single basic dye.

singlet oxygen Highly reactive molecular oxygen ($O_2^{\cdot -}$).

siRNA Small interfering RNA; An intermediate in the RNAi process in which the long double-stranded RNA has been cut up into short (~21 nucleotides) double-stranded RNA.

site-directed mutagenesis Techniques used to modify a gene in a specific location to produce the desired polypeptide.

slide agglutination test A method of identifying an antigen by combining it with a specific antibody on a slide.

slime layer A glycocalyx that is unorganized and loosely attached to the cell wall.

sludge Solid matter obtained from sewage.

smear A thin film of material containing microorganisms, spread over the surface of a slide.

smooth ER Endoplasmic reticulum without ribosomes.

SNP Single nucleotide polymorphism (pronounced "snip"). Single base-pair variations in the genomes of a population, found in at least 1% of the population.

snRNP Small nuclear ribonucleoprotein (pronounced "snurp"). Short RNA transcript plus protein that combines with pre-mRNA to remove introns and join exons together.

solute A substance dissolved in another substance.

solvent A dissolving medium.

Southern blotting A technique that uses DNA probes to detect the presence of specific DNA in restriction fragments separated by electrophoresis.

specialized transduction The process of transferring a piece of cell DNA adjacent to a prophage to another cell.

species The most specific level in the taxonomic hierarchy. *See also* bacterial species; eukaryotic species; viral species.

specific epithet The second or species name in a scientific binomial. *See also* species.

specificity Percentage of false positive results given by a diagnostic test.

spectrum of microbial activity The range of distinctly different types of microorganisms affected by an antimicrobial drug; a wide range is referred to as a broad spectrum of activity.

spheroplast A gram-negative bacterium treated to damage the cell wall, resulting in a spherical cell.

spicule One of two external structures on the male roundworm used to guide sperm.

spike A carbohydrate-protein complex that projects from the surface of certain viruses.

spiral *See* spirillum and spirochete.

spirillum (plural: **spirilla**) (1) A helical or corkscrew-shaped bacterium. (2) When written as a genus, refers to aerobic, helical bacteria with clumps of polar flagella.

spirochete A corkscrew-shaped bacterium with axial filaments.

spontaneous generation The idea that life could arise spontaneously from nonliving matter.

spontaneous mutation A mutation that occurs without a mutagen.

sporadic disease A disease that occurs occasionally in a population.

sporangiophore An aerial hypha supporting a sporangium.

sporangiospore An asexual fungal spore formed within a sporangium.

sporangium A sac containing one or more spores.

spore A reproductive structure formed by fungi and actinomycetes. *See also* endospore.

sporogenesis *See* sporulation.

sporozoite A trophozoite of *Plasmodium* found in mosquitoes, infective for humans.

sporulation The process of spore and endospore formation; also called sporogenesis.

spread plate method A plate count method in which inoculum is spread over the surface of a solid culture medium.

staining Colorizing a sample with a dye to view through a microscope or to visualize specific structures.

staphylococci (singular: **staphylococcus**) Cocci in a grapelike cluster or broad sheet.

stationary phase The period in a bacterial growth curve when the number of cells dividing equals the number dying.

stem cell An undifferentiated cell that gives rise to a variety of specialized cells.

stereoisomers Two molecules consisting of the same atoms, arranged in the same manner but differing in their relative positions; mirror images; also called D-isomer and L-isomer.

sterile Free of microorganisms.

sterilization The removal of all microorganisms, including endospores.

steroid A specific group of lipids, including cholesterol and hormones.

stipe A stemlike supporting structure of multicellular algae and basidiomycetes.

storage vesicle Organelles that form from the Golgi complex; contain proteins made in the rough ER and processed in the Golgi complex.

strain Genetically different cells within a clone. *See* serovar.

streak plate method A method of isolating a culture by spreading microorganisms over the surface of a solid culture medium.

streptobacilli (singular: **streptobacillus**) Rods that remain attached in chains after cell division.

streptococci (singular: **streptococcus**) (1) Cocci that remain attached in chains after cell division. (2) When written as a genus, refers to gram-positive, catalase-negative bacteria.

streptokinase A blood-clot dissolving enzyme, produced by beta-hemolytic streptococci.

streptolysin A hemolytic enzyme, produced by streptococci.

structural gene A gene that determines the amino acid sequence of a protein.

subacute disease A disease with symptoms that are intermediate between acute and chronic.

subclinical infection An infection that does not cause a noticeable illness; also called inapparent infection.

subcutaneous mycosis A fungal infection of tissue beneath the skin.

substrate Any compound with which an enzyme reacts.

substrate-level phosphorylation The synthesis of ATP by direct transfer of a high-energy phosphate group from an intermediate metabolic compound to ADP.

subunit vaccine A vaccine consisting of an antigenic fragment.

sulfhydryl group —SH.

sulfur cycle The various oxidation and reduction stages of sulfur in the environment, mostly due to the action of microorganisms.

sulfur granule See inclusion.

superantigen An antigen that activates many different T cells, thereby eliciting a large immune response.

superbug Bacterium resistant to a large number of antibiotics.

superficial mycosis A fungal infection localized in surface epidermal cells and along hair shafts.

superinfection The growth of a pathogen that has developed resistance to an antimicrobial drug being used; the growth of an opportunistic pathogen.

superoxide dismutase (SOD) An enzyme that destroys superoxide:
 $O_2^- + O_2^- + 2 H^+ \rightarrow H_2O_2 + O_2$

superoxide radical A toxic anion (O_2^-) with an unpaired electron.

surface-active agent Any compound that decreases the tension between molecules lying on the surface of a liquid; also called surfactant.

susceptibility The lack of resistance to a disease.

symbiosis The living together of two different organisms or populations.

symptom A change in body function that is felt by a patient as a result of a disease.

syncytium A multinucleated giant cell resulting from certain viral infections.

syndrome A specific group of signs or symptoms that accompany a disease.

synergism The principle whereby the effectiveness of two drugs used simultaneously is greater than that of either drug used alone.

synthesis reaction A chemical reaction in which two or more atoms combine to form a new, larger molecule.

synthetic drug A chemotherapeutic agent that is prepared from chemicals in a laboratory.

systematics The science organizing groups of organisms into a hierarchy.

systemic anaphylaxis A hypersensitivity reaction causing vasodilation and resulting in shock; also called anaphylactic shock.

systemic (generalized) infection An infection throughout the body.

systemic mycosis A fungal infection in deep tissues.

tachyzoite A rapidly growing trophozoite form of a protozoan.

T antigen An antigen in the nucleus of a tumor cell.

tapeworm A flatworm belonging to the class Cestoda.

target cell An infected body cell to which defensive cells of the immune system bind.

taxa Subdivisions used to classify organisms, e.g., domain, kingdom, phylum.

taxis Movement in response to an environmental stimulus.

taxonomy The science of the classification of organisms.

T cell A type of lymphocyte, which develops from a stem cell processed in the thymus gland, that is responsible for cell-mediated immunity. See also T cytotoxic cells, T helper cells, T regulatory cells.

TCRs (T cell receptors) Molecules on T cells that recognize antigens.

T cytotoxic (T_C) cells A precursor to a cytotoxic T lymphocyte.

T helper (T_H) cell A specialized T cell that often interacts with an antigen before B cells interact with the antigen.

T regulatory (T_{reg}) cells Lymphocytes that appear to suppress other T cells.

T-dependent antigen An antigen that will stimulate the formation of antibodies only with the assistance of T helper cells. See also T-independent antigen.

teichoic acid A polysaccharide found in gram-positive cell walls.

telomere Noncoding regions of DNA at the ends of eukaryotic chromosomes.

teleomorph The sexual stage in the life cycle of a fungus; also refers to a fungus that produces both sexual and asexual spores.

temperate phage A phage capable of lysogeny.

temperature abuse Improper food storage at a temperature that allows bacteria to grow.

terminator The site on a DNA strand at which transcription ends.

tertiary sewage treatment A method of waste treatment that follows conventional secondary sewage treatment; nonbiodegradable pollutants and mineral nutrients are removed, usually by chemical or physical means.

tetrad A group of four cocci.

thallus The entire vegetative structure or body of a fungus, lichen, or alga.

thermal death point (TDP) The temperature required to kill all the bacteria in a liquid culture in 10 minutes.

thermal death time (TDT) The length of time required to kill all bacteria in a liquid culture at a given temperature.

thermoduric Heat resistant.

thermophile An organism whose optimum growth temperature is between 50°C and 60°C; a heat loving microbe.

thermophilic anaerobic spoilage Spoilage of canned foods due to the growth of thermophilic bacteria.

thylakoid A chlorophyll-containing membrane in a chloroplast. A bacterial thylakoid is also known as a chromatophore.

thymus A mammalian organ responsible for maturation of the immune system.

thymic selection Elimination of T cells that don't recognize self antigens (major histocompatibility complex).

tincture A solution in aqueous alcohol.

T-independent antigen An antigen that will stimulate the formation of antibodies without the assistance of T helper cells. See also T-dependent antigen.

tinea Fungal infection of hair, skin, or nails.

Ti plasmid A tumor-inducing plasmid that can be incorporated into a host plant chromosome; found in *Agrobacterium*.

titer An estimate of the amount of antibodies or viruses in a solution; determined by serial dilution and expressed as the reciprocal of the dilution.

TLR (Toll-like receptor) Transmembrane protein of immune cells that recognizes pathogens and activates an immune response directed against those pathogens.

topoisomerase Enzyme that relaxes supercoiling of DNA ahead of replication fork; separates DNA circles at the end of DNA replication.

total magnification The magnification of a microscopic specimen, determined by multiplying the ocular lens magnification by the objective lens magnification.

toxemia The presence of toxins in the blood.

toxigenicity The capacity of a microorganism to produce a toxin.

toxin Any poisonous substance produced by a microorganism.

toxoid An inactivated toxin.

T plasmid An *Agrobacterium* plasmid carrying genes for tumor induction in plants.

trace element A chemical element required in small amounts for growth.

trans Hydrogen atoms on opposite side across a double bond in a fatty acid. See cis.

transamination The transfer of an amino group from an amino acid to another organic acid.

transcription The process of synthesizing RNA from a DNA template.

transduction The transfer of DNA from one cell to another by a bacteriophage. See also generalized transduction; specialized transduction.

transferrin One of several human iron-binding proteins that reduce iron available to a pathogen.

transfer RNA (tRNA) The type of RNA molecule that brings amino acids to the ribosomal site where they are incorporated into proteins.

transfer vesicle Membrane-bound sacs that move proteins from the Golgi complex to specific areas in the cell.

transformation (1) The process in which genes are transferred from one bacterium to another as “naked” DNA in solution. (2) The changing of a normal cell into a cancerous cell.

transient microbiota The microorganisms that are present in an animal for a short time without causing a disease.

translation The use of mRNA as a template in the synthesis of protein.

transmission electron microscope (TEM) An electron microscope that provides high magnifications (10,000–100,000 \times) of thin sections of a specimen.

transport media Media used to keep microorganisms alive between sample collection and laboratory testing; usually used for clinical samples.

transport vesicle Membrane-bound sacs that move proteins from the rough ER to the Golgi complex.

transporter protein A carrier protein in the plasma membrane.

transposon A small piece of DNA that can move from one DNA molecule to another.

trickling filter A method of secondary sewage treatment in which sewage is sprayed out of rotating arms onto a bed of rocks or similar materials, exposing the sewage to highly aerobic conditions and microorganisms.

triglyceride A simple lipid consisting of glycerol and three fatty acids.

triplex agent A short segment of DNA that binds to a target area on a double strand of DNA blocking transcription.

trophophase The period in the production curve of an industrial cell population in which the primary metabolites are formed; a period of rapid, logarithmic growth. *See also* idiophase.

trophozoite The vegetative form of a protozoan.

tuberculin skin test A skin test used to detect the presence of antibodies to *Mycobacterium tuberculosis*.

tumor necrosis factor (TNF) A polypeptide released by phagocytes in response to bacterial endotoxins.

tumor-specific transplantation antigen (TSTA) A viral antigen on the surface of a transformed cell.

turbidity The cloudiness of a suspension.

turnover number The number of substrate molecules acted on per enzyme molecule per second.

two-photon microscope A light microscope that uses fluorescent stains and long wavelength light.

ubiquinone A low-molecular weight, nonprotein carrier in an electron transport chain; also called coenzyme Q.

ultra-high-temperature (UHT) treatment A method of treating food with high temperatures (140–150°C) for very short times to make the food sterile so that it can be stored at room temperature.

uncoating The separation of viral nucleic acid from its protein coat.

undulating membrane A highly modified flagellum on some protozoa.

unsaturated A fatty acid with one or more double bonds.

use-dilution test A method of determining the effectiveness of a disinfectant using serial dilutions.

vaccination The process of conferring immunity by administering a vaccine; also called immunization.

vaccine A preparation of killed, inactivated, or attenuated microorganisms or toxoids to induce artificially acquired active immunity.

vacuole An intracellular inclusion, in eukaryotic cells, surrounded by a plasma membrane; in prokaryotic cells, surrounded by a proteinaceous membrane.

valence The combining capacity of an atom or a molecule.

vancomycin An antibiotic that inhibits cell wall synthesis.

variola An early method of vaccination using infected material from a patient.

vasodilation Dilation or enlargement of blood vessels.

VDRL test A rapid screening test to detect the presence of antibodies against *Treponema pallidum*. (VDRL stands for Venereal Disease Research Laboratory.)

vector (1) A plasmid or virus used in genetic engineering to insert genes into a cell. (2) An arthropod that carries disease-causing organisms from one host to another.

vegetative Referring to cells involved with obtaining nutrients, as opposed to reproduction.

vehicle transmission The transmission of a pathogen by an inanimate reservoir.

vertical gene transfer Transfer of genes from an organism or cell to its offspring.

vesicle (1) A small serum-filled elevation of the skin. (2) Smooth oval bodies formed in plant roots by mycorrhizae.

V factor NAD^+ or NADP^+ .

vibrio (1) A curved or comma-shaped bacterium. (2) When written as a genus (*Vibrio*), a gram-negative, motile, facultatively anaerobic curved rod.

viral hemagglutination The ability of certain viruses to cause the clumping of red blood cells in vitro.

viral hemagglutination inhibition test A neutralization test in which antibodies against particular viruses prevent the viruses from clumping red blood cells in vitro.

viral species A group of viruses sharing the same genetic information and ecological niche.

viremia The presence of viruses in the blood.

virion A complete, fully developed viral particle.

viroid Infectious RNA.

virology The scientific study of viruses.

virulence The degree of pathogenicity of a microorganism.

virus A submicroscopic, parasitic, filterable agent consisting of a nucleic acid surrounded by a protein coat.

volutin Stored inorganic phosphate in a prokaryotic cell. *See also* metachromatic granule.

Western blotting A technique that uses antibodies to detect the presence of specific proteins separated by electrophoresis.

whey The fluid portion of milk that separates from curd.

xenobiotics Synthetic chemicals that are not readily degraded by microorganisms.

xenodiagnosis A method of diagnosis based on exposing a parasite-free normal host to the parasite and then examining the host for parasites.

xenotransplantation product A tissue graft from another species; also called xenograft.

X factor Substances from the heme fraction of blood hemoglobin.

yeast Nonfilamentous, unicellular fungi.

yeast infection Disease caused by growth of certain yeasts in a susceptible host.

zone of inhibition The area of no bacterial growth around an antimicrobial agent in the disk-diffusion method.

zoonosis A disease that occurs primarily in wild and domestic animals but can be transmitted to humans.

zoospore An asexual algal spore; has two flagella.

zygospore A sexual fungal spore characteristic of the zygomycetes.

zygote A diploid cell produced by the fusion of two haploid gametes.

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See the connection between

**HUMAN HEALTH &
MICROBIOLOGY**

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